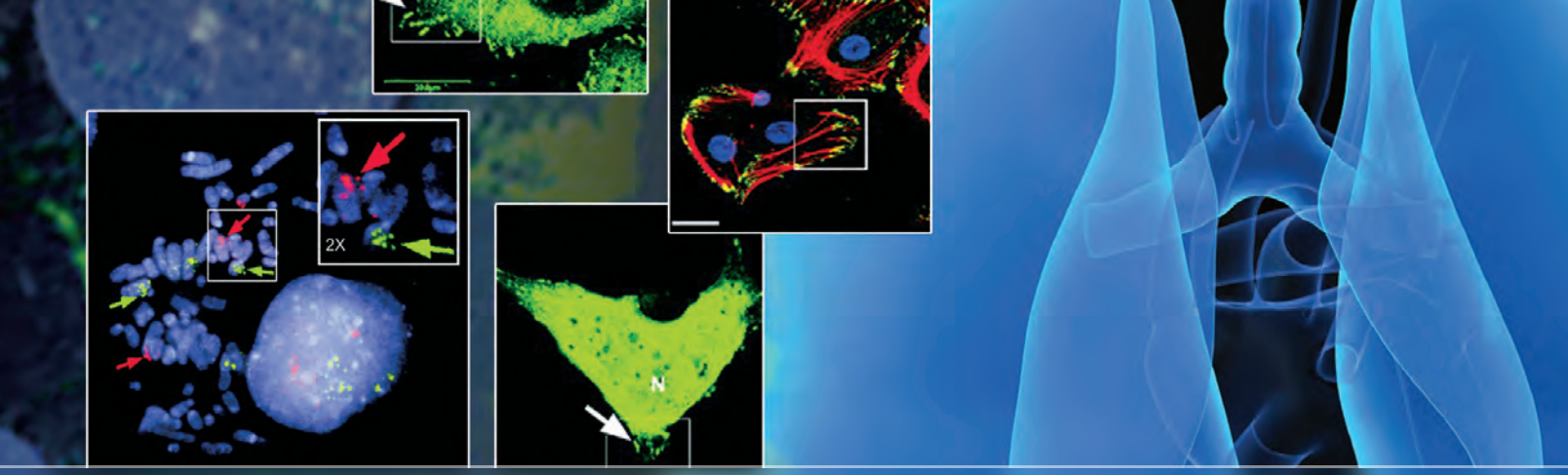

Sixth AACR-IASLC International Joint Conference

LUNG CANCER TRANSLATIONAL SCIENCE FROM THE BENCH TO THE CLINIC

January 11-14, 2020 | Marriot Marquis San Diego | San Diego, CA

CONFERENCE CHAIRS

John V. Heymach
The University of Texas MD Anderson Cancer Center, Houston, TX

Katerina A. Politi
Yale Cancer Center, New Haven, CT

CONFERENCE COCHAIRS

Trever G. Bivona
University of California San Francisco, San Francisco, CA

Christine M. Lovly
Vanderbilt University School of Medicine, Nashville, TN

Program and Proceedings

ABIM CME MOC ACCREDITED

Continuing Medical Education (CME) Activity–*AMA PRA Category 1 Credits*™ available

AACR American Association for Cancer Research®

IASLC

IASLC 2020
Meetings Schedule

Sixth AACR-IASLC International Joint Conference: Lung Cancer
January 11-14, 2020 | San Diego, CA | #Lung20

IASLC 2020 Targeted Therapies of Lung Cancer Meeting
February 19-22, 2020 | Santa Monica, CA | #TTLC20

FDA-AACR-IASLC Workshop to Address the Criticality of Tobacco Use Assessment in Oncology Therapeutic Trials
February 28, 2020 | Silver Springs, MD | #FDAWorkshop20

European Lung Cancer Congress 2020
April 15-18, 2020 | Geneva, Switzerland | #ELCC20

Lung Cancer Hot Topic: Liquid Biopsy
May 7-9, 2020 | Baltimore, MD | #LiquidBiopsy20

IASLC 2020 World Conference on Lung Cancer
August 9-12, 2020 | Singapore | #WCLC20

IASLC 2020 North America Conference on Lung Cancer
October 15-17, 2020 | Chicago, IL | #NACLC20

Lung Cancer Hot Topic: Immunotherapy
November 2020

CONNECT WITH US
@IASLC
facebook.com/IASLC
linkedin.com
IASLC

IASLC
INTERNATIONAL ASSOCIATION FOR THE STUDY OF LUNG CANCER
Conquering Thoracic Cancers Worldwide

Sixth AACR-IASLC International Joint Conference

LUNG CANCER TRANSLATIONAL SCIENCE FROM THE BENCH TO THE CLINIC

January 11-14, 2020 | Marriott Marquis San Diego | San Diego, CA

Conference Chairs

John V. Heymach
The University of Texas MD Anderson Cancer Center
Houston, TX

Katerina A. Politi
Yale Cancer Center
New Haven, CT

Conference Cochairs

Trever G. Bivona
University of California San Francisco
San Francisco, CA

Christine M. Lovly
Vanderbilt University School of Medicine
Nashville, TN


IASLC
2020 World Conference on Lung Cancer
AUGUST 9–12, 2020 | SINGAPORE
Registration will open January 2020.
#WCLC20
SAVE THE DATE
for the world's largest international gathering of clinicians, researchers and scientists in thoracic oncology.
wclc2020.IASLC.org
CONQUERING THORACIC CANCERS WORLDWIDE

TABLE OF CONTENTS

January 11, 2020

Dear Colleagues,

On behalf of the American Association for Cancer Research (AACR) and the International Association for the Study of Lung Cancer (IASLC), it is our pleasure to welcome you to the **Sixth AACR-IASLC International Joint Conference on Lung Cancer Translational Science from the Bench to the Clinic.**

We are very grateful to Conference Chairs Drs. John V. Heymach and Katerina A. Politi and Cochairs Drs. Trever G. Bivona and Christine M. Lovly for the time and dedication they have put into organizing this exciting and diverse program.

Continuing in the tradition of this biennial series that began in 2010, this conference will bring together a diverse group of attendees (physicians, patient advocates, and scientists in basic, translational, and clinical research) and provide a venue to discuss recent advances and establish new collaborations. This conference is not only a dynamic collaboration of the AACR and the IASLC, two organizations committed to the study of cancer, but also a dynamic collaboration of the many individuals charged with the study and treatment of this disease. We have an incredible roster of speakers and poster presentations of the most current research in the field, and we are thrilled to provide the opportunity for this scientific interaction.

The AACR and the IASLC extend our thanks to Novartis, Genentech, and Janssen for their support of this conference. We also thank AstraZeneca, Celgene, Novocure, and Pfizer for the Professional Educational Grants in support of this conference. Finally, we extend our thanks to the National Cancer Institute Center to Reduce Cancer Health Disparities for their generous support of travel awards for this meeting.

Once again, welcome to the Sixth AACR-IASLC International Joint Conference on Lung Cancer Translational Science from the Bench to the Clinic. We feel confident that you will find this to be an exciting and engaging meeting and look forward to your active participation.

Best wishes for a successful conference!

Sincerely,

Margaret Foti, PhD, MD (h.c.)
Chief Executive Officer, AACR

Dave Mesko, MBA
Chief Executive Officer, IASLC

Certificates of Attendance and Receipts

Certificates of attendance and receipts for conference registration fees are available at the AACR Registration Desk.

Meeting Room Locations

All conference locations are on Level One in the North Tower of the Marriott Marquis San Diego.

Conference Registration

Registration will be held at the AACR Registration Desk in the Pacific Ballroom Pre-Function Area 23-26 on Level One of the North Tower on the following schedule:

Saturday, January 11	3:00 p.m.-9:00 p.m.
Sunday, January 12	7:00 a.m.-5:00 p.m.
Monday, January 13	7:00 a.m.-6:00 p.m.
Tuesday, January 14	7:00 a.m.-12:30 p.m.

Internet

There will be complimentary basic wireless Internet in guest rooms and the General Session room, which allows for basic browsing and email.

Social Media

While we encourage your use of social media in and around AACR and IASLC conferences, we remind you to adhere to the AACR's and IASLC's social media guidelines and accepted social media etiquette. Please be aware of the following guidelines:

Do

- Follow us on Twitter @AACR and @IASLC and use the hashtag #Lung20 for this conference.
- Follow us on Facebook at facebook.com/aacr.org and facebook.com/IASLC.
- Blog about the conference and what you are hearing and seeing (but without sharing details of any data presented; follow journal rules about data sharing).
- Converse with other attendees.
- Provide feedback to AACR and IASLC staff and the program committee—discuss topics of interest and/or speakers for future conferences.
- Communicate with respect, being mindful of diversity and tolerant of differences you may encounter. Keep criticism constructive, and listen carefully to others to understand their perspectives.

Don't

- Engage in rudeness or personal attacks.

Meeting Policies and Procedures

- **Photography and Social Media Policies**
    - **Photography.** Conference attendees may take photographs during oral or poster presentations provided that the photographs are strictly for personal, noncommercial use and are not to be published in any form. Attendees are prohibited from using flash photography or otherwise distracting the presenters or members of the audience.
    - **Social Media.** Conference attendees may share information from presentations on social media provided that they respect the wishes of presenters. Oral presenters may label any or all slides in their presentations with "DO NOT POST." Similarly, poster presenters may label their posters with "DO NOT POST." Attendees must respect the presenters' requests in these instances and refrain from posting any images from these designated slides or posters on social media.
- In accordance with the Resolution adopted at the 1968 Annual Meeting of the AACR, registrants must refrain from smoking in all meeting rooms. This regulation applies to all session rooms, including the poster area.
- Children under 12 years of age are not permitted in any scientific session or poster session at any time. Children cannot be left unattended or unsupervised.
- Cell phones, pagers, and other electronic devices must be turned off or placed on "silent" mode before entering a session.
- Lost and found: Attendees may contact the Registration Desk for any lost items.

- Poster presenters are solely responsible for placing their poster on the assigned poster board and removing their poster according to the schedule provided. The AACR/IASLC cannot be responsible for any posters that are not removed at the designated time. Posters left in the Poster Hall after that time may be discarded.
- Poster presenters should not leave any items at their poster board unattended, including poster tubes, meeting bags, Programs, personal items, etc. The AACR/IASLC are not responsible for any items left in the Poster Hall.

Membership

AACR Membership

The AACR has more than 42,000 members in 120 countries and territories around the world. Over 30% of members live outside the United States and Canada, and 20% of the AACR's international members are located in countries with emerging economies. The AACR is a dynamic and vibrant organization that offers its members programs and activities that promote the exchange of timely scientific information, and excellent opportunities to participate more fully in the global conquest of cancer by fostering important relationships and collaborations with cancer scientists internationally. Six categories of membership in the AACR are available to support each aspect of our members' professional development and enhancement in cancer research. The AACR is also eager to support the exchange of knowledge and research with investigators who are located in countries with emerging economies. Significantly reduced membership dues are available for these investigators. Join our mission and apply for AACR membership today!

IASLC Membership

The International Association for the Study of Lung Cancer (IASLC) is the only global organization dedicated solely to the study of lung cancer and other thoracic malignancies. Founded in 1974, the association's membership includes more than 8,000 lung cancer specialists across all disciplines in over 100 countries, forming a global network working together to conquer lung and thoracic cancers worldwide.

Elimination of Annual Dues for AACR Associate Members (Predoctoral Students and Postdoctoral and Clinical Fellows)

The AACR fully supports the education, training and professional development of early-career investigators. Graduate students, medical students, residents and postdoctoral and clinical fellows who are enrolled in education or training programs that could lead to a career in cancer research are not required to pay annual membership dues. Membership is also available for undergraduates and high school students at no charge. Learn more and apply for Associate or Student membership.

AACR membership applications are available onsite. Simply review the information on the form and submit a completed application to AACR staff at the conference or send via email to membership@aacr.org. Candidates may also apply online at myaacr.aacr.org.

Poster Sessions

Poster Session A will be held in Pacific Ballroom 25/26 on Sunday, January 12, from 12:00 p.m.-2:00 p.m. Lunch will be provided. Poster Session B will be held in Pacific Ballroom 25/26 on Monday, January 13, from 5:00 p.m.-7:00 p.m. Light refreshments will be served.

Receptions and Meals

Continental Breakfast: Continental breakfast will be served Sunday, Monday, and Tuesday from 7:00 a.m.-8:00 a.m. in Pacific Ballroom 25/26. All attendees and registered guests are invited to attend. Conference badges are required.

Breaks: All breaks will be held in Pacific Ballroom Pre-Function Area 23-26 on the following schedule:

Saturday, January 11	6:00 p.m.-6:15 p.m.
Sunday, January 12	9:30 a.m.-10:00 a.m.
Sunday, January 12	4:00 p.m.-4:15 p.m.
Monday, January 13	10:00 a.m.-10:30 a.m.
Tuesday, January 14	10:00 a.m.-10:30 a.m.

Opening Reception: The Opening Reception will be held on Saturday, January 11, from 7:15 p.m.-9:15 p.m. in Pacific Ballroom 25/26. Hors d'oeuvres will be served and all attendees will receive one drink ticket. All attendees are invited to attend. Conference badges are required.

Join 8,000+ other thoracic oncology specialists from around the globe.

Learn about novel translational lung cancer science as it happens.

Become an IASLC Member

Here are just some of the benefits IASLC members enjoy:

- Scientific publications offering: member access to *IASLC Lung Cancer News/Journal of Thoracic Oncology*, discount publication fees for the new open-access *Journal of Thoracic Oncology Clinical and Research Reports*
- Networking opportunities within the ONLY global, multidisciplinary network dedicated to the study and eradication of thoracic malignancies
- Discounts to the IASLC World Conference on Lung Cancer and other IASLC-sponsored scientific and educational meetings
- Committee opportunities
- Access to the IASLC member directory

To learn more about the different levels of membership and benefits, visit: **www.iaslc.org/join**, visit the IASLC Booth or reach out to **membership@iaslc.org**

IASLC

INTERNATIONAL ASSOCIATION FOR THE STUDY OF LUNG CANCER

Conquering Thoracic Cancers Worldwide

The AACR and IASLC would like to thank the following organizations for their generous support of this conference.

Major Supporter

Novartis

Supporters

Genentech

Janssen

Professional Educational Grants

AstraZeneca
Celgene
Novocure
Pfizer

JTO Impact Factor increases to 12.460!

Ranked **10th** among **229** oncology journals

Ranked **3rd** among **63** respiratory medicine journals

JTO is the **#1** journal in thoracic malignancies, according to Editor-in-Chief, Alex A. Adjei, MD, PhD

IASLC

INTERNATIONAL ASSOCIATION FOR THE STUDY OF LUNG CANCER

Conquering Thoracic Cancers Worldwide

AACR Scholar-in-Training Awards

Four presenters of meritorious abstracts have been selected by the Conference Cochairs to receive awards to attend this conference. All graduate and medical students, postdoctoral fellows, and physicians-in-training who are AACR Associate Members and applied were eligible for consideration. The names of the Scholar-in-Training awardees, their affiliations, and the poster numbers are provided below.

Rui Li, University of California Los Angeles, Los Angeles, CA, A11

Erin Marshall, BC Cancer Research Centre, BC, Canada, A03

Fangfei Qu, Stanford University, Stanford, CA, B23

Adam Schoenfeld, Memorial Sloan Kettering Cancer Center, New York, NY, A07

Minority and Minority-Serving Institution Faculty Scholar in Cancer Research Award

Full-time minority faculty and faculty of Minority-Serving Institutions (Historically Black Colleges and Universities [HBCUs], Hispanic-Serving Institutions [HSIs], American Indian Tribally-Controlled Colleges and Universities [AITCCUs], and other postsecondary institutions as defined by the U.S. Department of Education) who present a proffered paper at this conference are encouraged to apply for this meritorious scholar award. Supported by a generous grant from the Center to Reduce Cancer Health Disparities of the National Cancer Institute, the purposes of these awards are to increase the scientific knowledge base of minority faculty and faculty at MSIs, to encourage them in their research, and to assist in inspiring their students to pursue careers in cancer research. Only citizens of the United States or Canada or scientists who are permanent residents of these countries may receive one of these awards.

Laura Riobolos, University of Washington, Seattle, WA, A34

CONTINUING MEDICAL EDUCATION (CME)

Accreditation Statement

The American Association for Cancer Research (AACR) is accredited by the Accreditation Council for Continuing Medical Education (ACCME) to provide continuing medical education activities for physicians.

Credit Designation Statement

AACR has designated this live activity for a maximum of 18.75 *AMA PRA Category 1 Credit(s)*™. Physicians should only claim credit commensurate with the extent of their participation in the activity.

Credit certification for individual sessions may vary, dependent upon compliance with the ACCME Accreditation Criteria. The final number of credits may vary from the maximum number indicated above.

Claiming CME Credit

Physicians and other health care professionals seeking *AMA PRA Category 1 Credit(s)*™ for this live continuing medical education activity must complete the online CME Request for Credit Survey by **Tuesday, Feb. 25, 2019.** Certificates will only be issued to those who complete the survey. The Request for Credit Survey will be available via a link on the AACR website at **AACR.org/Lung20cme** and via email. *Your CME certificate will be sent to you via email after the completion of the activity.*

Successful completion of this CME activity, which includes participation in the evaluation component, enables the participant to earn up to 18.75 Medical Knowledge MOC points in the American Board of Internal Medicine's (ABIM) Maintenance of Certification (MOC) program. Participants will earn MOC points equivalent to the amount of CME credits claimed for the activity. It is the CME activity provider's responsibility to submit participant completion information to ACCME for the purpose of granting ABIM MOC credit.

To receive ABIM MOC, participants must request MOC in the CME Request for Credit Survey and complete all questions. Once these steps are completed, AACR will submit your completion information via the ACCME's Program and Activity Reporting System for the purpose of granting MOC points.

Statement of Educational Need, Target Audience, and Learning Objectives

Lung cancer is the leading cause of cancer-related death in the United States and globally. In 2019, the estimated number of deaths from lung cancer in the United States (142,670) and worldwide (over 1.7 million people) is staggering.

In recent years, we have come to identify many of the genetic alterations that fuel lung cancer growth and drug resistance mechanisms. Major strides in harnessing the immune system to treat lung cancer have been made, increasing the number of treatment modalities in lung cancer. Identifying and verifying potential therapeutic targets, developing and testing therapeutics for optimal treatment, and evaluating mechanisms of resistance to therapy requires a multidisciplinary approach and collaborative efforts.

Notably, since the last conference in this series occurred in 2018 (between August 1, 2018 and July 31, 2019), three cell-signaling inhibitors (dacomitinib, lorlatinib, and the second approved tissue-agnostic drug, larotrectinib, for NTRK-positive solid tumors) and three immune checkpoint inhibitors (atezolizumab, nivolumab, and pembrolizumab) have been approved/approved for new use in certain types of lung cancer. The ability to discuss the data and propel progress forward on this new treatment front and emerging resistance to other treatment options will be possible in our conference forum.

Early detection remains critical in improving the survival rates in those diagnosed with cancer. The 5-year survival rate for lung cancer is 19%. Only 16% of lung cancer cases are diagnosed at a localized stage; however, the 5-year survival rate at this stage is significantly higher (56%). Technologies in early detection, such as continued recommendations for screening and the potential of liquid biopsies and circulating tumor DNA, also provide opportunities for collaboration of physicians and translational/basic research colleagues.

Bridging the gap between what physicians understand about cancer biology and its application to clinical oncology is critical to the implementation of the most current, approved molecular-based tests to aid in the diagnosis, treatment, and prevention of cancer. Moreover, facilitating the interface between physicians and scientists will increase physicians' knowledge of the epidemiologic implications of lung cancer incidence and the contributions of laboratory research to drug development and alternate strategies should a cancer become resistant to therapy.

After participating in this CME activity, physicians should be able to:

1. Demonstrate a knowledge of the current state of the field of lung cancer at various stages, including lung preneoplasia, tumor progression, and metastasis
2. Assess the current therapeutic options and mechanisms of action for targeting Ras-driven lung cancers
3. Compare and discuss current therapeutic modalities in lung cancer
4. Articulate currently studied genomic and nongenetic mechanisms of resistance to therapy

Disclosure Statement

It is the policy of the AACR that the information presented at AACR CME activities will be unbiased and based on scientific evidence. To help participants make judgments about the presence of bias, AACR will provide information that Scientific Program Committee members and speakers have disclosed about financial relationships they have with commercial entities that produce or market products or services related to the content of this CME activity. This disclosure information will be made available in the *Program/Proceedings* of this conference.

Acknowledgment of Financial or Other Support

This activity is supported by Professional Educational Grants from AstraZeneca, Celgene, Novocure, and Pfizer. Any others will be disclosed at the activity.

Questions about CME?

Please contact the Office of CME at 215-440-9300 or cme@aacr.org.

The
LungAmbition
Alliance

Accelerating advances
for people with lung cancer.

Together, we can break through limits.

There is only one way to solve the impossible. Together.

Imagine a world where every lung cancer patient is screened before the disease spreads. Where every patient gets the right drug at the right time, and the goal of any treatment plan is to cure. Where every patient, everywhere in the world, can expect to receive the highest quality care that provides the best odds in the fight against the disease. **Where no patient dies from lung cancer.**

Together, AstraZeneca, the International Association for the Study of Lung Cancer, the Global Lung Cancer Coalition and Guardant Health have come to form the Lung Ambition Alliance. We share one goal: to double long-term survival in lung cancer by 2025.

Through initiatives that increase early diagnosis, accelerate delivery of innovative precision medicines and improve quality care, we believe there is a way to solve the seemingly impossible and ultimately eliminate lung cancer as a cause of death.

Please visit LungAmbitionAlliance.org to know more about our first projects and help shape our priorities. Your insights will serve as an important first step on our life-changing journey toward accomplishing the impossible … together.

Join us at LungAmbitionAlliance.org.

AstraZeneca

IASLC

GLOBAL LUNG CANCER COALITION

GUARDANT

Date of preparation May 2019 Date of expiry May 2020 Document ID Z4-16997

AACR American Association for Cancer Research®

UPCOMING CONFERENCES AND WORKSHOPS

Advances in Liquid Biopsies
Conference Cochairs: Luis A. Diaz Jr., Maximilian Diehn, Irene M. Ghobrial, and Nicholas C. Turner
January 13-16, 2020 | Miami, FL

The Microbiome, Viruses, and Cancer
Conference Cochairs: Cynthia L. Sears, Giorgio Trinchieri, Jennifer A. Wargo, and Laurence Zitvogel
February 21-24, 2020 | Orlando, FL

EACR-AACR Basic and Translational Research Conference: Tumor Microenvironment
In partnership with ASPIC (Portuguese Association for Cancer Research)
Scientific Committee Cochairs: Carlos M. Caldas, Luís Costa, and Lisa M. Coussens
March 2-4, 2020 | Lisbon, Portugal

The Evolving Landscape of Cancer Modeling
Conference Cochairs: Cory Abate-Shen, Andrea Califano, Jos Jonkers, and Calvin J. Kuo
March 2-5, 2020 | San Diego, CA

Evolutionary Dynamics in Carcinogenesis and Response to Therapy
Conference Cochairs: James DeGregori, Marco Gerlinger, Robert Gillies, and Andriy Marusyk
March 12-15, 2020 | Denver, CO

Advances in Prostate Cancer Research
Conference Cochairs: Felix Y. Feng, Karen E. Knudsen, and Scott A. Tomlins
March 12-15, 2020 | Denver, CO

NIH-AACR Cancer, Autoimmunity, and Immunology Conference
Organizing Committee: Julie R. Brahmer, Elad Sharon, Connie Sommers, Howard Young, Ravi Madan, Katarzyna (Kasia) Bourcier, Marie Mancini, Annette Rothermel, and Lisa Spain
March 23-24, 2020 | Bethesda, MD

AACR Annual Meeting 2020
Program Committee Chair: Antoni Ribas
April 24-29, 2020 | San Diego, CA

Seventh JCA-AACR Special Joint Conference on the Latest Advances in Pancreatic Cancer Research: From Basic Science to Therapeutics
Organizing Committee: Kohei Miyazono, Masanobu Oshima, Hiroshi Seno, Elizabeth M. Jaffee, Anirban Maitra, and Rosalie C. Sears
June 9-11, 2020 | Kyoto, Japan

Second AACR International Meeting: Advances in Malignant Lymphoma: Maximizing the Basic-Translational Interface for Clinical Application
In cooperation with the International Conference on Malignant Lymphoma (ICML)
Scientific Committee Chair: Ari M. Melnick
June 25-28, 2020 | Boston, MA

Please visit
AACR.org/meetingcalendar
for additional conferences and program updates.

Saturday, January 11

4:30 p.m.-6:15 p.m. **PLENARY SESSION 1: THERAPEUTIC TARGETING AND VULNERABILITIES OF RAS-DRIVEN LUNG CANCER**
Pacific Ballroom 23-24

4:30 p.m.–4:55 p.m. **Title to be announced**
Ferdinandos Skoulidis, The University of Texas MD Anderson Cancer Center, Houston, TX

4:55 p.m.–5:20 p.m. **Insights into KRAS biology to identify potential therapeutic strategies**
Chiara Ambrogio, Dana-Farber Cancer Institute, Boston, MA

5:20 p.m.–5:45 p.m. **Therapeutic approaches in KRAS-driven non-small cell lung cancer**
Caroline McCoach, UCSF School of Medicine, San Francisco, CA

5:45 p.m.–6:00 p.m. **Transcriptional subtypes resolve tumor heterogeneity and identify therapeutic vulnerabilities in lung cancer***
Jonathan Cooper, Genentech, Inc., South San Francisco, CA

6:00 p.m.-6:15 p.m. **The SHP2 inhibitor RMC-4630 in patients with KRAS-mutant non-small cell lung cancer: Preliminary evaluation of a first-in-man phase 1 clinical trial***
Sai-Hong Ignatius Ou, University of California Irvine, Irvine, CA

6:15 p.m.-6:30 p.m. **BREAK**
Pacific Ballroom Pre-Function Area 23-26

6:30 p.m.-7:30 p.m. **WELCOME REMARKS AND OPENING KEYNOTE**
Pacific Ballroom 23-24

Welcome Remarks
Chandra P. Belani, Chief Scientific Officer, International Association for the Study of Lung Cancer

Early-stage drug development in the 21st century
William Pao, Roche Innovation Center Basel, Basel, Switzerland
(not eligible for CME credit)

7:30 p.m.-9:30 p.m. **OPENING RECEPTION**
Pacific Ballroom 25/26

**Short talk from proffered abstract*

CONFERENCE PROGRAM

Sunday, January 12

7:00 a.m.–8:00 a.m.	**CONTINENTAL BREAKFAST** Pacific Ballroom 25/26
8:00 a.m.–9:30 a.m.	**PLENARY SESSION 2: LUNG PRENEOPLASIA AND EARLY DETECTION** Pacific Ballroom 23-24
8:00 a.m.–8:25 a.m.	**Genomic underpinnings of tumor behavior in in situ and early lung adenocarcinoma** Pierre Massion, Vanderbilt-Ingram Cancer Center, Nashville, TN
8:25 a.m.–8:50 a.m.	**Intercepting lung cancer by understanding premalignant changes in the airway field** Jennifer E. Beane, Boston University School of Medicine, Boston, MA
8:50 a.m.–9:15 a.m.	**Plasma proteomic, glycomic, and autoantibody biomarkers for lung cancer early detection** Paul D. Lampe, Fred Hutchinson Cancer Research Center, Seattle, WA
9:15 a.m.–9:30 a.m.	**The genome-wide mutational landscape of lung cancer in never-smokers: The Women's Health Initiative (WHI) cohort*** Sitapriya Moorthi, Fred Hutchinson Cancer Research Center, Seattle, WA
9:30 a.m.–10:00 a.m.	**BREAK** Pacific Ballroom Pre-Function Area 23-26
10:00 a.m.–11:30 p.m.	**PLENARY SESSION 3: METABOLISM** Pacific Ballroom 23-24
10:00 a.m.–10:25 a.m.	**Mapping mitochondrial heterogeneity in lung cancer** David B. Shackelford, UCLA David Geffen School of Medicine, Los Angeles, CA
10:25 a.m.–10:50 a.m.	**Identification of new therapeutic targets in non-small cell lung cancer** Kathryn A. O'Donnell, UT Southwestern Medical Center, Dallas, TX
10:50 a.m.–11:15 a.m.	**Novel metabolic functions for redox regulators in non-small cell lung cancer** Gina M. DeNicola, H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL
11:15 a.m.–11:30 a.m.	**Integrated proteometabolomic analysis reveals metabolic vulnerabilities in small-cell lung cancer*** Antony Prabhu, H. Lee Moffitt Cancer Center, Tampa, FL

**Short talk from proffered abstract*

11:35 a.m. **POSTER SESSION A HIGHLIGHTS**
Pacific Ballroom 25/26

Presenters will give a 2-minute, 1-slide teaser preview of their posters to be presented in Poster Session A.

(not eligible for CME credit)

(A03) **Lung adenocarcinoma resident microbiome may contribute to cancer hypomethylation status**
Erin Marshall, BC Cancer Research Centre, Vancouver, BC, Canada

(A05) **ART1, a mono-ADP-ribosyltransferase, regulates tumor-infiltrating CD8+ T cells and is highly expressed in EGFR mutated lung cancers**
Sumit Mukherjee, Weill Cornell Medicine, New York, NY

(A06) **Tri-complex inhibitors of the oncogenic, GTP-bound form of KRASG12C overcome RTK-mediated escape mechanisms and drive tumor regressions in preclinical models of NSCLC**
Robert Nichols, Revolution Medicines, Redwood City, CA

(A07) **The genomic landscape of SMARCA4 alterations and association with patient outcomes in lung cancer**
Adam Schoenfeld, Memorial Sloan Kettering Cancer Center, New York, NY

(A08) **MYC-driven SCLC has unique metabolic vulnerabilities**
Sarah Wait, Huntsman Cancer Institute, Salt Lake City, UT

12:00 p.m.-2:00 p.m. **POSTER SESSION A / LUNCH**
Pacific Ballroom 25/26

2:00 p.m.-4:00 p.m. **PLENARY SESSION 4: PRECISION IMMUNOTHERAPY**
Pacific Ballroom 23-24

2:00 p.m.-2:25 p.m. **Role of the tumor microenvironment in sensitivity and resistance to immunostimulatory therapies in NSCLC**
Kurt A. Schalper, Yale University, New Haven, CT

2:25 p.m.-2:50 p.m. **Liquid biopsy approaches for precision immuno-oncology**
Valsamo K. Anagnostou, Sidney Kimmel Cancer Center at Johns Hopkins, Baltimore, MD

2:50 p.m.-3:15 p.m. **Targeting myeloid cells that define the tumor immune microenvironment in NSCLC**
Thomas U. Marron, Mt. Sinai Medical Center Tisch Cancer Institute, New York, NY

3:15 p.m.–3:40 p.m.	**Preclinical and translational approaches to capturing mechanisms of immunotherapy response and resistance in NSCLC** Don L. Gibbons, The University of Texas MD Anderson Cancer Center, Houston, TX
3:40 p.m.–3:55 p.m.	**A reservoir of tumor-specific CD8 T cells in lung cancer resides in the draining lymph node*** Nikhil Joshi, Yale University, New Haven, CT
4:00 p.m.–4:15 p.m.	**BREAK** Pacific Ballroom Pre-Function Area 23-26
4:15 p.m.–6:00 p.m.	**PLENARY SESSION 5: TARGETING TUMOR SUPPRESSORS AND "UNDRUGGABLE" TARGETS** Pacific Ballroom 23-24
4:15 p.m.–4:40 p.m.	**Decoding critical targets of LKB1/STK11 in NSCLC** Reuben J. Shaw, Salk Institute, La Jolla, CA
4:40 p.m.–5:05 p.m.	**A new generation of anti-Myc mini-proteins as potential therapy for NSCLC** Laura Soucek, Vall d'Hebron Institute of Oncology (VHIO), Barcelona, Spain
5:05 p.m.–5:30 p.m.	**Druggable vulnerabilities in therapy-resistant lung cancers** Kris C. Wood, Duke University, Durham, NC
5:30 p.m.–5:45 p.m.	**Blockade of myeloid suppressor cells overcomes the anti-PD-1/PD-L1 resistance in KRAS-driven and LKB1-deficient NSCLC*** Rui Li, University of California Los Angeles, Los Angeles, CA
5:45 p.m.–6:00 p.m.	**Proteogenomic characterization reveals therapeutic vulnerabilities in lung adenocarcinoma*** Michael Gillette, Broad Institute of MIT and Harvard, Cambridge, MA

**Short talk from proffered abstract*

Monday, January 13

7:00 a.m.–8:00 a.m.	**CONTINENTAL BREAKFAST** Pacific Ballroom 25/26
8:00 a.m.–9:30 a.m.	**PLENARY SESSION 6: CELLULAR THERAPIES, VACCINES, AND NEW IO MODALITIES** Pacific Ballroom 23-24
8:00 a.m.–8:25 a.m.	**A new world for lung cancer vaccines: Beyond picking a single antigen for everyone** Edward B. Garon, University of California (UCLA), Los Angeles, CA
8:25 a.m.–8:50 a.m.	**Evaluating the role of B cells and tertiary lymphoid structures in lung cancer development and progression** Tullia C. Bruno, University of Pittsburgh School of Medicine, Pittsburgh, PA
8:50 a.m.–9:05 a.m.	**Dendritic cell in situ vaccination potentiates anti-PD-1 efficacy and induces immunoediting in a murine model of NSCLC*** Ramin Salehi-Rad, University of California Los Angeles, Los Angeles, CA
9:05 a.m.–9:20 a.m.	**N-803 plus nivolumab for advanced or metastatic non-small cell lung cancer: Update on phase II experience of combination PD1 blockade with an IL-15 superagonist*** John Wrangle, Medical University of South Carolina, Charleston, SC
9:30 a.m.–10:00 a.m.	**BREAK** Pacific Ballroom Pre-Function Area 23-26
10:00 a.m.–12:20 p.m.	**PLENARY SESSION 7: SMALL-CELL LUNG CANCER** Pacific Ballroom 23-24
10:00 a.m.–10:25 a.m.	**Pan-cancer convergence to a small-cell neuroendocrine phenotype that shares susceptibilities with hematologic malignancies** Thomas G. Graeber, University of California Los Angeles, Los Angeles, CA
10:25 a.m.–10:50 a.m.	**ASCL1 represses a latent osteogenic program in small-cell lung cancer arising from multiple cells of origin** Trudy G. Oliver, University of Utah Huntsman Cancer Institute, Salt Lake City, UT
10:50 a.m.–11:15 a.m.	**Identifying chemically tractable vulnerabilities in small-cell lung cancer** David G. McFadden, UT Southwestern Medical Center, Dallas, TX
11:15 a.m.–11:40 a.m.	**Developing new therapies in small-cell lung cancer using parallel clinical and laboratory-based studies** Anna Farago, Massachusetts General Hospital, Boston, MA

**Short talk from proffered abstract*

CONFERENCE PROGRAM

11:40 a.m.–12:05 p.m. **Targeting DLL3 in small-cell lung cancer with novel modalities**
John T. Poirier, Perlmutter Cancer Center at NYU Langone Health, New York, NY

12:05 p.m.–12:20 p.m. **Unraveling the mechanisms of small-cell lung cancer brain metastasis***
Fangfei Qu, Stanford University School of Medicine, Stanford, CA

12:20 p.m.–2:00 p.m. **LUNCH ON OWN**

2:00 p.m.–3:15 p.m. **PLENARY SESSION 8: METASTASIS AND TUMOR PROGRESSION**
Pacific Ballroom 23-24

2:00 p.m.–2:25 p.m. **Adaptive determinants of metastatic progression in lung adenocarcinoma**
Don X. Nguyen, Yale University School of Medicine, New Haven, CT

2:25 p.m.–2:50 p.m. **Stage-specific roles of RB constrain tumor progression, lineage fidelity, and metastasis**
David Feldser, University of Pennsylvania, Philadelphia, PA

2:50 p.m.–3:15 p.m. **Restoring Capicua (CIC) expression to limit lung cancer metastasis**
Ross A. Okimoto, University of California San Francisco, San Francisco, CA

3:20 p.m.–4:50 p.m. **Panel Discussion: What to Do about Squamous Cell?**
Pacific Ballroom 23-24

Panel Moderator: Trudy G. Oliver, University of Utah Huntsman Cancer Institute, Salt Lake City, UT

Panelists:
Eric B. Haura, H. Lee Moffitt Cancer Center & Research Institute, Tampa, FL
Trudy G. Oliver
Paul Paik, Memorial Sloan Kettering Cancer Center, New York, NY
Kwok-Kin Wong, New York University Langone Medical Center, New York, NY

Targeting glucose reliance in lung squamous cell carcinoma*
Jung-whan Kim, University of Texas at Dallas, Richardson, TX

4:55 p.m. **POSTER SESSION B HIGHLIGHTS**
Pacific Ballroom 25/26

Presenters will give a 2-minute, 1-slide teaser preview of their posters to be presented in Poster Session B.

(not eligible for CME credit)

(B01) **Active YAP as a functional marker of drug-tolerant persister cells in EGFR-mutant and ALK fusion-positive NSCLC**
Franziska Haderk, University of California San Francisco, San Francisco, CA

**Short talk from proffered abstract*

(B02) **The GSK3 signaling axis regulates adaptive glutamine metabolism in lung squamous cell carcinoma**
Milica Momcilovic, University of California Los Angeles, Los Angeles, CA

(B03) **JNJ-61186372, an Fc effector enhanced EGFR/cMet bispecific antibody, induces EGFR/cMet downmodulation and efficacy through monocyte and macrophage trogocytosis**
Sheri Moores, Janssen Research & Development, Spring House, PA

(B04) **Integrative approach to map the tumor suppressor landscape of small-cell lung cancer genome**
Kwon-Sik Park, University of Virginia, Charlottesville, VA

(B05) **Identifying SCLC vulnerabilities using phenotypic chemical screens**
Juan Manuel Povedano Selfa, University of Texas Southwestern Medical Center, Dallas, TX

(B06) **Time-resolved RNA-seq identifies transient gene expression changes following initial chemotherapy challenge in small-cell lung cancer**
David Shia, University of California Los Angeles, Los Angeles, CA

(B07) **Mechanisms of alectinib resistance in a leptomeningeal carcinomatosis of EML4-ALK lung cancer and its circumvention by EGR-TKIs**
Seiji Yano, Kanazawa University, Kanazawa, Ishikawa, Japan

5:15 p.m.-7:15 p.m. **POSTER SESSION B / RECEPTION**
Pacific Ballroom 25/26

Tuesday, January 14

7:00 a.m.-8:00 a.m. **CONTINENTAL BREAKFAST**
Pacific Ballroom 25/26

8:00 a.m.-10:00 a.m. **PLENARY SESSION 9: GENOMIC MECHANISMS OF RESISTANCE**
Pacific Ballroom 23-24

8:00 a.m.-8:25 a.m. **Title to be announced**
Robert C. Doebele, University of Colorado Denver, Aurora, CO

8:25 a.m.-8:50 a.m. **Title to be announced**
Zofia Piotrowska, Massachusetts General Hospital, Boston, MA

CONFERENCE PROGRAM

8:50 a.m.-9:15 a.m.	**Investigating and overcoming primary resistance of EGFR and HER2 (ERBB2) exon 20 mutant NSCLC** Jacqulyne Robichaux, The University of Texas MD Anderson Cancer Center, Houston, TX
9:15 a.m.-9:35 a.m.	**Genetic contributors to tumor progression and drug resistance in EGFR mutant lung cancer** Katerina A. Politi, Yale Cancer Center, New Haven, CT
9:35 a.m.-9:45 a.m.	**Advocate presentation** Jill Feldman, Co-Founder, EGFR Resisters
9:45 a.m.-9:55 a.m.	**Advocate presentation** Janet Freeman-Daily, Co-Founder, The ROS1ders
10:00 a.m.-10:30 a.m.	**BREAK** Pacific Ballroom Pre-Function Area 23-26
10:30 a.m.-12:15 p.m.	**PLENARY SESSION 10: NONGENETIC MECHANISMS OF RESISTANCE** Pacific Ballroom 23-24
10:30 a.m.-10:55 a.m.	**Nongenetic mechanisms of resistance** Trever G. Bivona, University of California San Francisco, San Francisco, CA
10:55 a.m.-11:20 a.m.	**Mechanisms of small-cell lineage transformation in resistance to targeted therapies** William W. Lockwood, British Columbia Cancer Agency, Vancouver, BC, Canada
11:20 a.m.-11:45 a.m.	**The YAP/FOXM1 axis regulates EMT-associated EGFR tyrosine kinase inhibitor resistance and increased expression of spindle assembly checkpoint components** John V. Heymach, The University of Texas MD Anderson Cancer Center, Houston, TX
11:45 a.m.-12:05 p.m.	**Bypass signaling pathways that confer resistance to EGFR and ALK inhibitors** Christine M. Lovly, Vanderbilt University School of Medicine, Nashville, TN
12:05 p.m.-12:15 p.m.	**Advocate presentation** Colin Barton, Executive Board Member, ALK Positive

IA02 Insights into KRAS biology to identify potential therapeutic strategies. C. Ambrogio. University of Torino and Dana-Farber Cancer Institute, Torino, Italy.

Mutations in KRAS are among the most frequent RAS alterations in human cancers and the prevalent driver event in lung adenocarcinoma (LUAD). There are still no effective targeted therapies for KRAS-driven LUAD, although specific KRAS G12C inhibitors are showing very promising results in clinical trials. Small-molecule inhibitors of the MAPK pathway, one of the prominent downstream KRAS mediators, showed minimal clinical activity either as single agents or in combination with chemotherapy. We observed that loss of wild-type KRAS enhances tumor fitness in KRAS mutant cancer cells while concomitantly increasing sensitivity to MEK inhibition. Given the challenges of reanalyzing prior clinical trials, future clinical studies of targeted inhibitors should evaluate and/or stratify patients based on the relative expression of wild-type and mutant KRAS alleles to determine their correlation with treatment outcome. We also showed that dimerization/oligomerization between KRAS proteins is a key regulator for lung adenocarcinoma biology and determinant of treatment response. We generated an inducible system to force either wild-type/mutant or mutant/mutant KRAS dimerization, which showed that forced dimerization between wild-type/mutant KRAS resulted in impaired cell growth as compared to forced mutant/mutant KRAS dimerization.

Summary: Loss of wild-type KRAS enhances tumor fitness in KRAS mutant cancer cells while concomitantly increasing sensitivity to MEK inhibition. Dimerization of wild-type KRAS with mutant KRAS results in growth inhibition and changes the therapeutic index for MEK inhibitors. Mutant-mutant KRAS dimerization is critical for the full oncogenic properties of mutant KRAS. Collectively these observations suggest that strategies designed to interfere with KRAS dimerization should be evaluated as a therapeutic approach in KRAS mutant cancers.

IA03 Therapeutic approaches in KRAS-driven non-small cell lung cancer. C. E. McCoach. University of California San Francisco, San Francisco, CA.

RAS mutations (KRAS, HRAS, and NRAS) are the most common oncogenic drivers in non-small cell lung cancer (NSCLC). In metastatic NSCLC, KRAS mutations are associated with worse overall survival compared with KRAS wild-type tumors. They are also unique among the targetable alterations in NSCLC in that they are often associated with a patient smoking history. Though targeted therapies have led to significant improvements in survival of NSCLC patients with activating alterations in EGFR, ALK, ROS1, and BRAF, effective therapies targeting the RAS pathway have been elusive. The challenge in targeting KRAS reflects the complex biology of the RAS signaling pathway. KRAS proteins are membrane-bound effector proteins that link cell surface receptors to downstream growth and proliferation pathways. KRAS proteins are cytosolic protein that are linked to the cell membrane. They cycle between an inactive GDP-bound form and an active GTP-bound form with high affinity. Cycling between active and inactive states is regulated by guanine nucleotide exchange factors (GEFs) and GTPase activating proteins (GAPs). When constitutively active, such as in KRAS mutated NSCLC, overlapping downstream growth and proliferation pathways such as PI3K/ AKT, RAF/MEK/ERK, and RALGOS/RAL/RLBP1 become activated. Part of the challenge in blocking the oncogenic signaling pathways that originate from KRAS mutations is the crosstalk and redundancy within the pathway. Additionally, the comutational landscape of KRAS mutated NSCLC impacts responses to treatment and can have independent oncogenic activity, further adding to the challenge of blocking oncogenic signaling. Numerous therapeutic tactics have attempted to target this signaling pathway; however, until recently there has been limited success. Therapeutic approaches for KRAS-positive tumors include 1) targeting of the membrane attachment of the KRAS protein, 2) direct targeting of KRAS and its coactivation partners, 3) targeting of downstream and parallel growth and activation pathways, 4) targeting of synthetic lethal interactions, and 5) utilization of immunotherapy. Within and between each of these categories there are also combination therapies being developed. Despite the inherent complexity in developing treatments for KRAS mutated NSCLC, there are now multiple promising strategies in development that may change the treatment landscape of this disease. In this session, we will explore the background and current landscape of the therapeutic approaches for KRAS mutated NSCLC.

IA04 Early-stage drug development in the 21st century. W. Pao. Roche Innovation Center Basel, Basel, Switzerland.

For centuries, physicians have been developing and using instruments to characterize, classify, and measure aspects of human health and disease. Such tools have been vital to the development of

novel therapies. Concurrently, the study of "disease outliers" with "extreme phenotypes" as determined by such instruments has been a powerful approach to understand mechanisms of disease. Today, we are using a plethora of new tools such as smartphone apps, wearables, artificial intelligence, etc., that allow for "precision phenotyping." The new ability to collect data on each patient across their journey at unprecedented depth, combined with the ability to generate data across large patient populations, i.e., "meaningful data at scale," enables a more precise understanding of disease, disease activity, and response or resistance to therapy. Such efforts will lead to next-generation biologic insights, new drug targets, enhanced diagnostic and prognostic methods, new clinical endpoints, and ultimately the development of future breakthrough medicines that improve how patients feel, function, or survive.

IA05 Genomic underpinnings of tumor behavior in in situ and early lung adenocarcinoma. J. Qian[1], S. Zhao[1], Y. Zou[1], J. Rahman[1], M. Senosain[1], T. Stricker[1], C. Powell[2], A. Borczuk[3], P. Massion[1]. [1]Vanderbilt-Ingram Cancer Center, Nashville, TN, [2]Mount Sinai, New York, NY, [3]Weill Cornell, New York, NY.

Our understanding of the molecular underpinnings of early adenocarcinoma (ADC) progression remains limited. We hypothesized that the behavior of early ADC can be predicted based on genomic underpinnings. Objectives: To identify genomic alterations associated with resected indolent and aggressive early lung ADCs. DNA was extracted from 21 adenocarcinoma in situ (AIS), 27 minimally invasive adenocarcinoma (MIA), and 54 fully invasive adenocarcinoma and subjected to deep next-generation sequencing to target a custom 347-cancer gene panel. The associations between tumor mutation burden, frequencies of mutation and copy number alterations, mutation signatures, intratumor heterogeneity, pathway alterations, and histology as well as overall survival were performed. We found that deleterious mutation burden was significantly greater in invasive ADC. Intratumor heterogeneity occurs as early as in AIS. More copy number loss was observed in AIS/MIA. Twenty-one significantly mutated genes were shared among three groups. Mutation signature profiling had no significant difference among three groups, although APOBEC signature was associated with ADC subgroup and poor survival. Mutations of KRAS, TP53, and NF1 were found at an increasing frequency from AIS/MIA to ADC. A cancer progression model revealed selective early and late drivers. Subclonal KRAS mutations and a gene signature consisting of PIK3CG, ATM, EPPK1, EP300, or KMT2C mutations were associated with poor survival. Our results demonstrate several sequences of genetic driver events, gene clonality, and mutated gene signatures associated with outcome in early lung ADC with potential future implications in the management of early ADC.

IA06 Intercepting lung cancer by understanding premalignant changes in the airway field. J. Beane. Boston University School of Medicine, Boston, MA.

Lung cancer is the leading cause of cancer death. In order to decrease mortality, we need innovative strategies to intercept cancer development by diagnosing the disease at its earliest and potentially most curable stage. Development of lung cancer risk biomarkers and interception strategies requires a detailed understanding of the earliest molecular alterations involved in lung carcinogenesis that occur in the respiratory epithelium. Exposure to cigarette smoke creates a field of injury throughout the entire respiratory tract by inducing a variety of genomic alterations that can lead to an at-risk airway where lung squamous premalignant lesions develop. Lung squamous cell carcinoma arises in the epithelial layer of the bronchial airways and is often preceded by the development of premalignant lesions through a stepwise histologic progression from normal epithelium to hyperplasia, squamous metaplasia, dysplasia (mild, moderate, and severe), carcinoma in situ, and finally to invasive cancer. The presence of high-grade persistent or progressive dysplasia is a marker of increased lung cancer risk, although many lesions have varied outcomes. Recent molecular profiling of endobronchial biopsies representing a range of histologic scores revealed an upregulation of cell cycle, proliferation, and DNA repair pathways and downregulation of inflammation and immune-associated pathways in high-grade progressive/persistent lesions. This work provided a foundation with which to further our understanding of the mechanisms that drive these early alterations and to develop robust biomarkers to detect their presence and future behavior. Recent projects that build upon this work to elucidate the pathways responsible for histologic progression will be presented. These projects will be put into the context of the collaborative effort to create a lung precancer atlas that will include large-scale genomic, immunogenomic, and clinical multidimensional data. This larger effort will serve to both validate existing observations and biomarkers and further enhance lung cancer interception efforts.

IA07 Plasma proteomic, glycomic, and autoantibody biomarkers for lung cancer early detection. K. L. Lastwika[1], Y. Zhang[1], M. Shipley[1], P. E. Kinahan[2], S. Pipovath[2], V. Wu[2], P. P. Massion[3], A. M. Houghton[1], P. D. Lampe[1]. [1]Fred Hutchinson Cancer Research Center, Seattle, WA, [2]University of Washington, Seattle, WA, [3]Vanderbilt University, Nashville, TN.

Lung cancer is the leading cause of cancer deaths worldwide, with >159,000 deaths annually in the US alone. Making matters worse, five-year survival rates remain a dismal ~18%, since most lung cancer cases are identified at an advanced stage, which confers a poor prognosis. The National Lung Screening Trial (NLST) employed low-dose computed tomography (CT) imaging to screen for lung cancer in a high-risk population (smokers aged 55-74) and demonstrated a 20% reduction in mortality. These and other results led the US Preventative Services Task Force (USPSTF) to recommend CT screening for 55- to 80-year-old, 30-pack-year smokers. Unfortunately, screening uptake has been poor and pulmonary nodules are relatively common in this group compared to the incidence of cancer, leading to potentially avoidable radiation exposure, morbidity, and mortality effects. Also, CT performs best for detection of lung adenocarcinoma (LUAD) and less well for other subtypes. In line with several other groups, we propose that plasma biomarkers could help sort which nodules are malignant. Our approach combines proteomic, glycomic, and autoantibody plasma measures along with CT semantic and radiomic features to evaluate nodules particularly of the indeterminate size range (6-30 mm). Furthermore, we have found specific markers that predict or detect squamous cell carcinoma (LUSQ) and small cell lung cancer (SCLC)—subtypes that are less frequently found via CT. We have combined two novel approaches to improve risk stratification for subjects with pulmonary nodules. The first is based on a large-format antibody array we created containing >3,200 different antibodies to interrogate prediagnostic plasma sample sets for cancer early detection biomarkers. We utilize the same antibody array platform for proteomic, glycomic, and autoantibody-antigen interrogation by implementing three distinct probing strategies. Using prediagnostic lung cancer case and control specimens from the Cardiovascular Health Study (CHS), we found 68 proteins were upregulated in cases ($p<0.02$). Ten of these were also upregulated ($p<0.05$) in a validation set of malignant and benign nodules collected at the FHCRC Lung Cancer Early Detection and Prevention Clinic (LCEDPC). For glycomic analysis, of 9 and 8 proteins with higher sialyl-Lewis A (i.e., CA19-9) and sialyl-Lewis X levels that met stringent selection criteria, 2 and 2 proteins, respectively, were validated in the LCEDPC samples. For antibody-antigen analysis, of 81 and 44 proteins bound to IgG and IgM, 25 and 4 antigens, respectively, were validated in the LCEDPC samples. Differentiating non-small cell lung cancer (NSCLC) samples into LUAD or squamous cell carcinoma (LUSQ) generated a 4-marker LUAD-specific panel with an AUC of 0.82 in CHS and 0.87 in LCEDP and a LUSQ-specific panel had an AUC of 0.94 in CHS and 0.91 in LCEDPC. The second approach is to analyze both semantic and radiomic CT features and combine them with the plasma biomarkers. Using Lasso regression analysis to choose features from the validated set of plasma markers, we found 5 semantic, 2 radiomic, and 8 plasma markers yielded an AUC>0.95 in LCEDPC samples from people with indeterminate (6-30 mm) nodules that we are now testing in an additional cohort. Since CT screening approaches capable of early detection for NSCLC have not proved effective for SCLC, we examined autoantibody-antigen levels that can distinguish case from control and found them >2x higher in SCLC compared to other cancer types, including NSCLC, colon, breast, and pancreas cancer. Using high-density antibody arrays, we discovered and twice validated 9 IgG and 12 IgM highly specific autoantibodies for SCLC in cohorts from the CHS, Vanderbilt, and the LCEDPC (total N=240). Using optimized logistic regression, we identified 4 autoantibody-antigen complexes with fixed coefficients (average AUC, 0.86) that performed well in each study. 4/4 panel autoantibodies were similarly effective when the plasma was drawn up to 1 year prior to diagnosis, at limited-stage or extensive-stage diagnosis and 2/4 were upregulated when the plasma was drawn up to 2 years prior to diagnosis. We have evidence that each panel autoantibody is specific for SCLC as none are upregulated in NSCLC (N=59) samples or in other comorbidities examined, including COPD (N=31) and autoimmunity (N=15). Our vision is that using blood drawn at the time of lung cancer screening, one could more definitively assign indeterminate nodules to different treatment paths (e.g., none, repeat CT, biopsy) and indicate when further imaging workup might be appropriate for potential cases of LUSQ or SCLC.

IA08 Mapping mitochondrial heterogeneity in lung cancer. M. Momcilovic[1], M. Han[1], E. Bushong[2], L. Stiles[1], S. M. Dubinett[1], H. Christofk[3], O. Shirihai[1], C. M. Koehler[4], S. Sadeghi[5], M. Ellisman[2], D. B. Shackelford[1]. [1]UCLA David Geffen School of Medicine, Los Angeles, CA, [2]University of California at San Diego, La Jolla, CA, [3]UCLA Department of Biological Chemistry, Los Angeles,

CA, [4]UCLA Department of Chemistry and Biochemistry, Los Angeles, CA, [5]UCLA Department of Pharmacology, Los Angeles, CA.

Non-small cell lung cancer (NSCLC) is a histologically, genetically, and metabolically heterogeneous disease. The mitochondria are essential regulators of cellular energy and metabolism, and they play a critical role in sustaining growth and survival of lung tumor cells. However, our understanding of mitochondrial metabolism in cancer at an in vivo level has been limited, thus leaving a large gap in our knowledge of how mitochondrial bioenergetics support tumor growth. To better study mitochondrial bioenergetics in lung tumors, we recently developed and validated a voltage-sensitive, positron emission tomography (PET) tracer known as 4-[18F]fluorobenzyl triphenylphosphonium (18F-BnTP) that we used to profile mitochondrial bioenergetics in autochthonous K-Ras driven mouse models of lung cancer. The use of 18F-BnTP PET imaging enabled us to functionally profile mitochondrial bioenergetics in live tumors and discover distinct functional mitochondrial heterogeneity conserved across different NSCLC tumor subtypes. In order to study mitochondria at the level of ultrastructure, we coupled 18F-BnTP PET with 3D serial block-face scanning electron microscopy (3D SBEM). By coupling these two techniques, we are able to image and quantify mitochondria heterogeneity from whole tumors down to the ultrastructures of individual mitochondria within tumor cells. Our study reveals distinct organization of mitochondrial structure and function as lung tumors adapt during tumorigenesis. We anticipate that coupling 18F-BnTP PET imaging with 3D SEM will have dynamic applications beyond that of lung cancer and enrich our understanding of how mitochondria impact human disease.

IA09 Identification of new therapeutic targets in non-small cell lung cancer. K. A. O'Donnell. UT Southwestern Medical Center, Dallas, TX.

Our laboratory is focused on understanding the mechanisms that contribute to tumor initiation, progression, and metastasis. Using unbiased forward genetic screens, we have identified novel genes that promote transformation of human bronchial epithelial cells (HBECs) and contribute to lung cancer pathogenesis. This approach enabled our discovery of novel oncogenic cell surface receptors in non-small cell lung cancer that may represent new therapeutic targets. For example, we recently identified the Transmembrane Serine Protease TMPRSS11B as a gene that promotes transformation of immortalized HBECs. TMPRSS11B is upregulated in human lung squamous cell cancers (LSCC), and high expression is associated with poor survival of non-small cell lung cancer patients. TMPRSS11B inhibition in human LSCCs reduced transformation and tumor growth. Given that TMPRSS11B harbors an extracellular protease domain, we hypothesized that catalysis of a membrane-bound substrate accelerates tumor progression. Interrogation of a set of soluble receptors revealed that TMPRSS11B promotes solubilization of Basigin, an obligate chaperone of the lactate monocarboxylate transporter MCT4. Basigin release mediated by TMPRSS11B enhanced lactate export and glycolytic metabolism, thereby promoting tumorigenesis. These findings established an oncogenic role for TMPRSS11B and provided support for the development of therapies that target this enzyme at the surface of cancer cells. Our latest results related to TMPRSS11B and other cell surface proteins in lung cancer will be presented. Together, these studies illustrate the power of unbiased forward genetic screening approaches to identify new oncogenic pathways and potential therapeutic targets in human malignancies.

IA10 Novel metabolic functions for redox regulators in non-small cell lung cancer. G. M. DeNicola. H. Lee Moffitt Cancer Center, Tampa, FL.

Redox regulators are emerging as critical mediators of lung tumorigenesis. Notably, NRF2 and its negative regulator KEAP1 are commonly mutated in human lung cancers. These mutations lead to NRF2 accumulation and constitutive expression of NRF2 target genes, many of which are at the interface of antioxidant function and anabolic processes that support cellular proliferation. However, much of our understanding about the regulation of, and requirement for, these metabolic alterations comes from studies in cell culture. To understand the deregulation of cellular metabolism by NRF2 in vivo, we generated genetically engineered, conditional murine alleles of the NRF2D29H and KEAP1R554Q mutations found in human NSCLC, and generated lung tumor models harboring these mutations. Our data from these models suggest that not all NRF2-regulated metabolic alterations are favorable and that NRF2 activation results in metabolic liabilities that must be overcome during tumorigenesis. While NRF2 primarily supports the cytoplasmic antioxidant system, the mitochondrial antioxidant system is also critical to mitigate the reactive oxygen species (ROS) generated as a byproduct of the robust and complex mitochondrial metabolism characteristic of lung tumors. Nicotinamide nucleotide transhydrogenase

(NNT) is known to sustain mitochondrial antioxidant capacity through the generation of NADPH; however, its function in non-small cell lung cancer (NSCLC) has not been established. To determine the importance of NNT activity to lung tumorigenesis, we studied lung tumor mice lacking NNT. We found that NNT expression significantly enhanced tumor formation as well as tumor aggressiveness in mouse models of lung tumor initiation and progression. Interestingly, while NNT significantly contributed to the NADPH:NADP+ ratio in lung cancer cell lines, NNT loss did not lead to global oxidative stress. Rather, NNT supported the activities of enzymes containing iron-sulfur (Fe-S) clusters, including aconitase and the electron transport chain subunits. Collectively, our work demonstrates the importance of redox regulators to lung tumor biology and uncovers distinct metabolic states arising from their perturbation.

IA11 Role of the tumor microenvironment in sensitivity and resistance to immunostimulatory therapies in NSCLC. K. A. Schalper. Yale University, New Haven, CT.

Effective anticancer immunostimulatory therapies can overcome dominant immune regulatory signals, restore tumor recognition, and achieve clinically meaningful/sustained tumor-cell killing. In most solid malignancies, the tumor microenvironment contains multiple and sometimes overlapping tolerogenic signals that suppress immune function and favor tumor progression. Understanding the immune composition and dominant tolerogenic pathways in lung cancer can support the identification of predictive biomarkers and guide the design of novel therapeutic modalities. This presentation will address current knowledge about the composition, molecular context, and functional tumor-immune interactions in human lung carcinomas. The presentation also includes discussion of novel approaches to analyze the tumor microenvironment using high multiplexed/spatially resolved methods and machine learning strategies.

IA13 Targeting myeloid cells that define the tumor immune microenvironment in NSCLC. T. U. Marron, A. Leader, Y. Lavin, B. Maier, M. Casanova-Acebes, A. Wolf, R. Flores, M. Beasley, A. Rahman, E. Kenigsberg, M. Merad. Tisch Cancer Institute, New York, NY.

The composition of the tumor immune microenvironment dictates responsiveness to cancer immunotherapies, though discrete biomarkers to predict responsiveness are yet to be defined. Much of the focus of the field of biomarker research has been on the spatial distribution and activation status of CD8 T cells when defining whether a tumor is "inflamed" and potentially responsive to immunotherapy. However, the majority of the leukocyte composition of most tumors, including non-small cell lung cancer (NSCLC), is of myeloid, not lymphoid, origin, and these cells appear to play a role in dictating therapeutic efficacy. Through single-cell analysis at the proteomic and transcriptomic level we can define the resident myeloid populations within the tumor, to determine the role these subsets play in developing an immunosuppressive tumor microenvironment, and identify potential therapeutic targets. Through mass cytometry our group has demonstrated selective depletion of resident alveolar macrophages, with enrichment of a distinct tumor-associated macrophage (TAM) population within the tumor. Single-cell RNA sequencing confirms the disparate transcriptome of these TAMs contrasted with resident lung macrophages, and direct concordance between a defined monocyte-derived TAM and T regulatory cell enrichment, as well as T effector cell dysfunction. These inhibitory myeloid subsets present a potential therapeutic target to further potentiate current immunotherapy approaches. We have identified multiple chemokine and cytokine pathways that may be integral to recruitment and maintenance of this immunosuppressive milieu, and validated dependence on these pathways in preclinical studies. To investigate the role of two of these myeloid-recruitment pathways in vivo in humans, we have designed a neoadjuvant "window-of-opportunity" trial that will evaluate the synergy of PD-1 blockade with disruption of the CCR2/5 or interleukin-8 mediated myeloid recruitment and retention within early-stage NSCLC lesions.

IA14 Preclinical and translational approaches to capturing mechanisms of immunotherapy response and resistance in NSCLC. D. L. Gibbons. The University of Texas MD Anderson Cancer Center, Houston, TX.

Strategies incorporating immune checkpoint inhibition have achieved unprecedented successes and been rapidly incorporated into standard-of-care regimens for patients with locally advanced or metastatic non-small cell lung cancer. Unfortunately, high rates of primary or acquired therapeutic resistance limit their broader efficacy for patients or durability. Using preclinical models, we have studied response and resistance to both single-agent and combination checkpoint blockade strategies. Consistently we have observed that the initial therapeutic response is accompanied by an overall reprogramming of the cellular immune

microenvironment, followed by the development of resistance. Upregulation of molecules such as CD38 on tumor cells and cells of the myeloid compartment orchestrate changes in the metabolic environment as well as the cellular landscape, each of which define important and targetable components of resistance. We have similarly built patient cohorts and interventional trials for patients who are treated with surgical resection to leverage the neoadjuvant treatment space for tissue-based examination of response and resistance. This approach allows us to monitor for clinical response or resistance to treatment, while obtaining appropriate tissues for deep, multiplatform profiling of the tumor, normal tissues, and circulating factors. These efforts include the ICON Project (ImmogenomiC PrOfiling of Non-small cell lung cancer), which has profiled 150 patient tumors without neoadjuvant treatment or with neoadjuvant chemotherapy only and longitudinally followed patients for outcomes. Additionally, in the phase II NEOSTAR trial patients received neoadjuvant nivolumab (n=23) or nivolumab plus ipilimumab (n=21) before undergoing surgical resection, with tumors from both of the arms undergoing multiplatform profiling. These individual efforts and comparison of the tumor data between them allow us to understand the baseline immunogenomic profiles and heterogeneity of NSCLC, as well as the effects of standard chemotherapy or immunotherapy. Using these datasets, we can define subsets of patients likely to respond to therapy, while identifying types of responses, biomarkers, and potential mechanisms that define resistance that can be targeted by combination or sequential therapies.

IA15 Decoding critical targets of LKB1/STK11 in NSCLC. R. J. Shaw. Salk Institute, San Diego, CA.

Inactivating mutations in the LKB1 (STK11) tumor suppressor are the third most frequent genetic alteration in non-small cell lung cancer (NSCLC). LKB1 encodes a serine/threonine kinase that directly phosphorylates and activates 14 members of the AMP-activated protein kinase family. The function of many of the AMPK-related kinases (AMPKRs) remains obscure, and which are most critical to the tumor-suppressive function of LKB1 remains unknown. Recently we have combined CRISPR and genetic analysis of the AMPKR family in NSCLC cell lines and mouse models, revealing multiple surprises. First, despite an unwavering role in inhibiting mTOR progrowth signaling, loss of AMPK at initiation in Kras GEMMs results in a block in tumor progression, which we could connect to a loss of lysosome and metabolic adaptive capability. Moreover, we found a surprising critical role for the SIK subfamily. Conditional genetic loss of Sik1 revealed increased tumor growth in mouse models of Kras -dependent lung cancer, which was further enhanced by loss of the related kinase Sik3. As most known direct substrates of SIK1 and SIK3 control transcription, gene-expression analysis was performed, revealing specific transcriptional programs that contribute to LKB1-dependent tumorigenesis. Additional pathways by which one might therapeutically target these tumors based on the signaling and metabolic pathways dysregulated from LKB1-deficiency will be discussed.

IA16 A new generation of anti-Myc mini-proteins as potential therapy for NSCLC. Marie-Eve Beaulieu[1,2], Toni Jauset[1,2], Daniel Massó-Vallés[1,2], Sandra Martínez-Martín[2], Mariano F. Zacarias-Fluck[2], Sílvia Casacuberta-Serra[1,2], Erika Serrano del Pozo[2], Laia Foradada[1], Virginia Castillo Cano[2], Génesis Martín[2], Jastrinjan Kaur[2], Miguel Ángel Morcillo Alonso[3], Jonathan R. Whitfield[2], Pierre Lavigne[4], Laura Soucek[1,2,5,6]. [1]Peptomyc S.L., Edifici Cellex, Hospital Vall d'Hebron, Barcelona, Spain, [2]Vall d'Hebron Institute of Oncology (VHIO), Edifici Cellex, Hospital Vall d'Hebron, Barcelona, Spain, [3]Centro de Investigaciones Energéticas, Medioambientales y Tecnológicas (CIEMAT), Madrid, Spain, [4]Département de Biochimie, PROTÉO and Institut de Pharmacologie de Sherbrooke, Université de Sherbrooke, Sherbrooke, QC, Canada, [5]Institució Catalana de Recerca i Estudis Avançats (ICREA), Barcelona, Spain, [6]Department of Biochemistry and Molecular Biology, Universitat Autònoma de Barcelona, Bellaterra, Spain.

MYC is one of the most wanted targets for therapeutic intervention in cancer, having a key role in driving and maintaining most, if not all, human tumors, including lung cancer. Despite this indisputable therapeutic opportunity, MYC has long been perceived as "undruggable" for its intrinsically disordered nature and fear of catastrophic side effects in normal tissues. Indeed, to date, there is still no MYC inhibitor in the clinic.

We previously designed a dominant negative form of MYC called Omomyc and used its conditional transgenic expression to inhibit MYC function both in vitro and in vivo, demonstrating a potent therapeutic impact in various mouse models of cancer, including non-small cell lung cancer (NSCLC), causing only mild, well-tolerated, and reversible side effects. Importantly, we recently showed that the purified Omomyc mini-protein displays unexpected cell-penetrating properties and can be used by direct tissue delivery or systemic administration to target NSCLC harboring different oncogenic mutation

profiles, indicating its potential for clinical development for the treatment of NSCLC patients. Clinical trials testing this use are due to begin in 2020.

IA17 Druggable vulnerabilities in therapy-resistant lung cancers. K. C. Wood. Duke University, Durham, NC.

Oncogene-targeted therapies often drive therapeutic responses in patients with advanced lung cancers, but frequently these responses eventually give way to acquired resistance. Blocking acquired resistance is a substantial and open-ended challenge whose difficultly is underscored by the fact that resistance within individual patients is often polyclonal in nature, driven by diverse and co-occurring mechanisms. Here, I will discuss two broad approaches we are employing in the search for therapeutic strategies that delay or circumvent resistance evolution. In the first area, we have identified vulnerabilities present in tumors at minimal residual disease states. For example, we have identified a molecular pathway through which targeted therapies such as EGFR, ALK, and BRAF inhibitors trigger double-strand DNA breaks in the cancer cells that comprise the minimal residual disease state. These cells rely upon an ATM-dependent DNA repair process for their survival and are thus hypersensitive to ATM inhibition. As such, combining oncogene-targeted therapies with an ATM inhibitor leads to more penetrant and durable responses to these agents in vivo. In the second area, we have identified vulnerabilities that arise specifically in tumor cells that develop acquired resistance to oncogene-targeted therapies. Importantly, we have identified scenarios in which these "collateral sensitivities" are conserved across heterogeneous resistant clones with distinct resistance mechanisms, implying that targeting these mechanisms may simultaneously eradicate diverse clones. I will describe examples of mechanism-based collateral sensitivities we have uncovered in lung cancers, melanomas, and leukemias, then demonstrate that by targeting these mechanisms in the upfront setting, it is possible to construct combination therapies that select against resistance.

IA18 A new world for lung cancer vaccines: Beyond picking a single antigen for everyone. E. B. Garon. David Geffen School of Medicine at UCLA, Los Angeles, CA.

Immunotherapy was hypothesized as an effective approach for the treatment of lung cancer for decades. Until the last decade, this enthusiasm was met with the cold reality of clinical trials, showing no benefit in engaging the immune system to fight lung cancer. This string of disappointing clinical trials included several high-profile trials of vaccines targeting a single, specific antigen. While enthusiasm for this approach of vaccines targeting a specific antigen has been reinvigorated in the era of PD-1 and PD-L1 inhibitors (generally in combination with these agents), new approaches to vaccines are also emerging. These new approaches often use patient-specific factors, such as autologous antigen presenting cells or vaccines directed at antigens specific to the patient being treated.

IA19 Evaluating the role of B cells and tertiary lymphoid structures in lung cancer development and progression. Tullia C. Bruno. University of Pittsburgh School of Medicine, Pittsburgh, PA.

Lung cancer is the leading cause of cancer death in both the United States and the world. Even with the best current treatments, the 5-year survival is only 15%. Immunotherapy has been impressively successful in multiple solid tumors, including non-small cell lung cancer (NSCLC), which, until recently, was always considered to be immune quiescent. Blockade of the inhibitory PD1:PDL1 pathway on CD8+ and CD4+ tumor-infiltrating lymphocytes (TILs) has revolutionized standard of care for NSCLC patients. Anti-PD1 can specifically target tumor cells without harming normal lung epithelial cells, which ultimately allows for fewer adverse events compared to standard chemotherapy or radiation. However, these approaches do not work in 80% of NSCLC patients; thus, a better understanding of the immune response prior to the development of cancer (heavy smokers and patients with chronic obstructive pulmonary disease [COPD]) compared to active disease (adenocarcinoma [LUAD] and squamous cell carcinoma [LUSC]) is necessary to develop new therapeutic approaches to enhance the antitumor immune response but also to assemble additional noninvasive, accurate screening methods for patients.

The current immunotherapies for NSCLC patients do not consider or target B cells despite their predominance in the tumor microenvironment (TME) and key role in the adaptive immune response. Further, in NSCLC patients, current evidence suggests an antitumor role for B cells as they can generate tumor-specific antibodies, present antigens to CD4+ TILs, and are detected within tertiary lymphoid structures (TLS), which also correlate with better prognosis. TLS predominantly contain B cells, CD4+ T conventional cells, and CD14+ myeloid cells; however, unlike normal lymphoid tissues, i.e.,

lymph node or tonsil, TLS in cancer patients do not always have well-defined germinal centers (GCs). GCs are paramount for proper B-cell development and function. Thus, in order to successfully implement B-cell targeting into future immunotherapies, we must increase our understanding of B-cell function in TLS within premalignancy and overt cancer. We hypothesize that B cells help generate potent, long-term, immune responses against lung tumor cells by educating CD4+ T cells in TLS and producing tumor-specific antibodies.

Toward this hypothesis, we have evaluated B cells and TLS in premalignancy and overt cancer via single-cell RNA sequencing, advanced spectral cytometry (Cytek Aurora), and multispectral imaging (Vectra and Nanostring GeoMx platforms). These analyses have revealed key differences in B-cell infiltration and TLS formation as lung cancer develops and progresses. We have utilized our results to create an objective signature for TLS identification. Specifically, we have observed an increase in GC-like TLS as patients develop cancer. We have also begun to evaluate the ex vivo function of B cells in patient tumors via antigen presentation and antibody production assays. We have evidence for a differential function for B cells within the TME that correlates with activation status. Since B cells and TLS are great prognostic indicators in NSCLC patients, an improved objective measure of the different tiers of these structures and how they correlate with disease progression could offer new and viable (a) biomarkers to predict lung cancer progression, (b) targets for early immunotherapeutic intervention in COPD patients that might trigger better antitumor immunity as patients develop lung cancer, and (c) immunotherapeutic targets in patients with already established NSCLC.

IA20 Pan-cancer convergence to a small-cell neuroendocrine phenotype that shares susceptibilities with hematologic malignancies. N. G. Balanis, K. M. Sheu, O. N. Witte, T. G. Graeber. University of California at Los Angeles, Los Angeles, CA.

Small-cell neuroendocrine (SCN) cancers are an aggressive cancer subtype. Transdifferentiation toward an SCN phenotype has been reported as a resistance route in response to targeted therapies. This has important consequences in that SCN cancers, once considered rare in many tissue types, may become increasingly common with the emergence of resistance cases. Here, we identified a molecular convergence to an SCN state that is more widespread across various epithelial cancers than previously realized, with these additional cases associated with poor prognosis. More broadly, non-SCN metastases have higher expression of SCN-associated transcription factors than non-SCN primary tumors. Drug sensitivity and gene dependency screens demonstrate that these convergent SCN cancers have shared vulnerabilities. These common vulnerabilities are found across unannotated SCN-like epithelial cases, pediatric small round blue cell tumors, and unexpectedly in hematologic malignancies. The SCN convergent phenotype and common sensitivity profiles with hematologic cancers can guide treatment options beyond the limitations of tissue-specific targeted therapies.

IA21 ASCL1 represses a latent osteogenic program in small-cell lung cancer arising from multiple cells of origin. R. R. Olsen[1], D. W. Kastner[1], A. S. Ireland[1], K. Pozo[2], C. P. Whitney[1], M. R. Guthrie[1], S. J. Wait[1], D. Soltero[1], B. L. Witt[1], A. Gazdar[2], J. E. Johnson[2], T. G. Oliver[1]. [1]University of Utah, Salt Lake City, UT, [2]University of Texas Southwestern Medical Center, Dallas, TX.

Small-cell lung cancer (SCLC) has been treated in the clinic as a single disease, but our previous work demonstrated that MYC drives a unique molecular and therapeutically relevant subset of SCLC (Mollaoglu et al., Cancer Cell 2017; Chalishazar et al., Clin Can Res 2019). Four major molecular subsets of SCLC have now been identified, and they are associated with high expression of four key developmental transcription factors: ASCL1, NEUROD1, POU2F3, and YAP1 (Rudin et al., Nat Rev Can 2019). ASCL1 is a lineage-specific oncogenic driver of SCLC, highly expressed in a significant fraction of tumors, that is required for the development of SCLC in specific mouse models. However, ~20% of human SCLC are ASCL1-low and associated with a non-neuroendocrine fate and high MYC expression. The role of ASCL1 in the MYC-driven subset of SCLC is unknown. Using genetically engineered mouse models (GEMMs), we show that alterations in Rb1/Trp53/Myc can drive SCLC in multiple cell types of origin and that these tumors initially express ASCL1. Genetic depletion of ASCL1 in MYC-driven SCLC dramatically inhibits tumor initiation but, surprisingly, converts tumors to an RUNX2+ osteogenic cell fate. Thus, ASCL1 normally represses the osteogenic fate in MYC-driven SCLC arising from multiple cells of origin. MYC-driven SCLC harbors gene signatures that resemble neural crest and mesenchymal stem cells, which have the cell fate options of becoming neuroendocrine or bone. These data suggest that ASCL1 is critical for neuroendocrine tumor cell fate even when initiated in non-neuroendocrine cells. Together, specific genetic alterations can promote remarkable plasticity or deprogramming of adult

lung cells, with ASCL1 repressing the emergence of nonendodermal tumor fates.

IA22 Identifying chemically tractable vulnerabilities in small-cell lung cancer. D. McFadden. UT Southwestern Medical Center, Dallas, TX.

Small-cell lung cancer is a clinically aggressive neuroendocrine cancer. Genome sequencing studies have failed to reveal frequent somatic mutations in genes encoding proteins that are targetable with currently available therapeutics. We have developed a series of tumor cell lines derived from genetically engineered mouse models of cancer (GEMMs) and performed a phenotypic small-molecule screen to identify SCLC/neuroendocrine-selective anticancer toxins. We will present preliminary results from this screening campaign, and recently developed methods used for identification of the molecular targets of small molecules identified from this and other HTS studies.

IA23 Developing new therapies in small-cell lung cancer using parallel clinical and laboratory-based studies. A. F. Farago. Massachusetts General Hospital Cancer Center, Boston, MA.

Small-cell lung cancer (SCLC) is an aggressive high-grade neuroendocrine malignancy with high metastatic potential and poor clinical outcomes. My translational research program utilizes both preclinical studies and clinical trial strategies to develop improved treatments for patients with SCLC. Preclinical and clinical studies have demonstrated activity of poly (ADP-ribose) polymerase (PARP) inhibitors in SCLC, though overall the activity of PARP inhibitor monotherapy has been quite modest. Combinations with other DNA-damaging agents have shown greater potential in trials. We conducted a phase I/II trial of combination olaparib tablets and temozolomide in previously treated SCLC. We established a recommended phase 2 dose (RP2D) of olaparib 200 mg PO BID with temozolomide 75 mg/m2 daily, both on days 1-7 of a 21-day cycle, and expanded to a total of 50 patients. The confirmed overall response rate (ORR) was 41.7% (20/48 evaluable), median progression-free survival (mPFS) was 4.2 months (95% CI 2.8-5.7), and median overall survival (mOS) was 8.5 months (95% CI 5.1-11.3) after a median follow-up of 7.1 months (Farago et al., Cancer Discovery 2019, PMID 31416802). Overall, these results indicate promising activity of combination olaparib and temozolomide in SCLC. In parallel, we have generated a panel of patient-derived xenograft (PDX) models of SCLC, using both tissue biopsies and circulating tumor cells (Drapkin et al., Cancer Discovery 2018, PMID 29483136). This panel includes 6 PDX models derived from patients enrolled to the olaparib/temozolomide trial. The responses of these in vivo tumor models to olaparib/temozolomide recapitulated the clinical responses of the corresponding patients. This enabled a coclinical trial in 32 PDX models, which we then utilized to identify putative biomarkers of response and resistance to olaparib/temozolomide. Using paired-end transcriptome sequencing, we identified a correlation between low basal expression of inflammatory response genes and cross-resistance to both olaparib/temozolomide and standard first-line chemotherapy, etoposide/platinum. We are now exploring mechanisms of acquired resistance to olaparib/temozolomide using serially derived PDX models from patients before and after treatment with this regimen. Updated data will be presented at the meeting.

IA24 Targeting DLL3 in small-cell lung cancer with novel modalities. J. T. Poirier. New York University Langone Health, New York, NY.

Delta-like ligand 3 (DLL3) is single-pass transmembrane Notch ligand that interacts with full-length, unprocessed NOTCH1 in the Golgi apparatus, inhibiting the pathway in cis. DLL3 is selectively overexpressed in the subtype of small-cell lung cancer (SCLC) driven by the transcription factor ASCL1 (SCLC-A) that accounts for 70% percent of diagnoses (95% CI [60–79]) (1). In one study immunoreactivity was observed in 1,040/1,363 (70.4%) of SCLC specimens, consistent with this incidence (2). Overexpression of DLL3 leads to low-level cell surface expression of the protein on the order of 10,000 proteins per cell while expression in normal tissues is restricted to intracellular compartments: the same study demonstrated only low to moderate cytoplasmic or nuclear immunoreactivity in normal adult tissues (3). High expression of DLL3 has also been reported in low-grade glioma (4,5), neuroendocrine prostate (6), and occasionally in other cancer types when neuroendocrine features are present (7,8). The exquisitely selective expression of surface DLL3 on cancer cells presents an excellent target for a variety of therapeutic strategies.

Rovalpituzumab teserine (Rova-T; SC16LD6.5) is an antibody-drug conjugate consisting of a monoclonal antibody targeting DLL3, a cathepsin-cleavable linker, and a pyrrolobenzodiazepine (PBD) warhead (4). The first-in-human clinical trial of Rova-T in recurrent

SCLC demonstrated encouraging activity despite often severe side effects attributable to the PBD warhead (9); however, the phase 2 TRINITY study showed a disappointing 16% objective response rate while reporting a similar toxicity profile (NCT02674568). Subsequently, the phase 3 TAHOE study was halted due to shorter overall survival in the treatment arm. A phase 3 trial of Rova-T in the maintenance setting (MERU) was terminated at the interim analysis due to lack of survival benefit (NCT03033511). AbbVie has discontinued development of Rova-T.

Other DLL3-targeting therapies under active investigation include the bispecific T-cell engager (BiTE) AMG757 (NCT03319940) and a chimeric antigen receptor CAR-T AMG119 (NCT03392064). These agents have shown significant antitumor activity in preclinical models of SCLC; however, AMG119 required direct delivery of the engineered T cells for activity. AMG575 was therefore the more potent of the two molecules and may be better suited to overcome known barriers to CAR-T activity in solid tumors.

Alternative strategies remain under exploration including the use of 89Zr-SC16, a PET radiotracer, for in vivo imaging and as a companion diagnostic to optimize the selection of patients for treatment with DLL3-directed therapeutic agents. 89Zr-labeled-SC16 antibody successfully delineated normal tissue from subcutaneous and orthotopic SCLC tumor xenografts. Radiotracer accumulation in tumors was directly correlated with the degree of DLL3 expression and also correlated with response to SC16LD6.5 therapy in SCLC patient-derived xenograft models.

On the basis of these preclinical results, an investigator-initiated first-in-human phase 1/2 clinical trial of 89Zr-SC16 was recently opened to determine the safety and feasibility of immunoPET imaging of DLL3 in patients with small-cell lung cancer.

References

1. Rudin CM et al. Molecular subtypes of small cell lung cancer: A synthesis of human and mouse model data. Nat Rev Cancer 2019;19:289-97, doi:10.1038/s41568-019-0133-9. 2. Huang RS P. et al. Delta-like protein 3 prevalence in small cell lung cancer and DLL3 (SP347) assay characteristics. Arch Pathol Lab Med 2019, doi:10.5858/arpa.2018-0497-OA. 3. Sharma SK et al. Non-invasive interrogation of DLL3 expression in metastatic small cell lung cancer. Cancer Res 2017, doi:10.1158/0008-5472.CAN-17-0299. 4. Saunders LR et al. A DLL3-targeted antibody-drug conjugate eradicates high-grade pulmonary neuroendocrine tumor-initiating cells in vivo. Sci Transl Med 2015;7:302ra136, doi:10.1126/scitranslmed.aac9459. 5. Spino M et al. Cell surface Notch ligand DLL3 is a therapeutic target in isocitrate dehydrogenase-mutant glioma. Clin Cancer Res 2019;25:1261-71, doi:10.1158/1078-0432.CCR-18-2312. 6. Puca L et al. Delta-like protein 3 expression and therapeutic targeting in neuroendocrine prostate cancer. Sci Transl Med 2019;11, doi:10.1126/scitranslmed.aav0891. 7. Koshkin VS et al. Transcriptomic and protein analysis of small-cell bladder cancer (SCBC) identifies prognostic biomarkers and DLL3 as a relevant therapeutic target. Clin Cancer Res 2019;25:210-21, doi:10.1158/1078-0432.CCR-18-1278. 8. Ding X, Li F, Zhang L. Knockdown of Delta-like 3 restricts lipopolysaccharide-induced inflammation, migration and invasion of A2058 melanoma cells via blocking Twist1-mediated epithelial-mesenchymal transition. Life Sci 2019;226:149-55, doi:10.1016/j.lfs.2019.04.024. 9. Rudin CM et al. Rovalpituzumab tesirine, a DLL3-targeted antibody-drug conjugate, in recurrent small-cell lung cancer: A first-in-human, first-in-class, open-label, phase 1 study. Lancet Oncol 2017;18:42-51, doi:10.1016/S1470-2045(16)30565-4.

IA25 Adaptive determinants of metastatic progression in lung adenocarcinoma. S. Adua, E. Wingrove, K. Patel, Z. Liu, D. Nguyen. Yale School of Medicine, New Haven, CT.

The central nervous system (CNS) is a major site of treatment refractory metastases from lung cancers, yet deciphering the mechanisms of brain relapse remains a challenge because of the complexity of the brain tumor microenvironment (TME) and the perceived pharmacologic limitations of systemic therapies. To define the molecular landscape of brain metastases in situ, we developed a bulk RNA sequencing-based approach (BMX-seq), which leverages the transcriptome of tumor xenografts and effectively distinguishes tumor cell and stromal gene expression with increased accuracy and sensitivity. BMX-seq analysis was also integrated with single-cell profiling of distinct metastasis cell populations. In models of metastatic non-small cell lung cancer, we demonstrate that tumor cells in the brain exhibit an enhanced capacity for resistance to targeted therapies, despite strong brain penetrance of drug. Accordingly, BMX-seq reveals shifts in cytoskeletal signaling, metabolic stress, and neuronal-like lineage programs in tumor cells as they adapt to the TME and the reciprocal neuroinflammatory response of the stroma. Several transcriptional hallmarks of metastasis are identified that are specific to particular regions of the brain and confirmed in syngeneic models and

patient biopsies. Finally, certain epigenetic alterations can be reversed, while others are features of selected tumor cell populations. Despite recent improvements in the pharmacologic properties of targeted therapies, drug resistance in the CNS still develops. Our results suggest that adaptive epigenetic responses to the brain TME not only promote malignant outgrowth but also precondition disseminated tumor cells for subsequent therapeutic responses.

IA26 Stage-specific roles of RB constrain tumor progression, lineage fidelity, and metastasis. D. M. Feldser. University of Pennsylvania, Philadelphia, PA.

Mutations in the Rb tumor suppressor pathway are a hallmark of cancer and a prevalent feature of lung adenocarcinoma. Additionally, recent clinical successes with cyclin-dependent kinase inhibitors have reinvigorated interest in reactivating the Retinoblastoma (Rb) pathway to treat lung adenocarcinoma and other tumor types. Remarkably, though, Rb's role in suppressing lung adenocarcinoma remains unclear and whether Rb pathway reactivation would be efficacious in this disease remains unknown. To model Rb pathway reactivation as a treatment strategy in lung adenocarcinoma and to shed light on its role in this disease, we established an Rb^{XTR} allele that enables Cre-dependent inactivation of Rb in developing tumors and allows Flp recombinase-inducible reactivation of Rb after tumors are established. In the $Kras^{Lox\text{-}Stop\text{-}Lox\text{-}G12D/+}$;$p53^{flox/flox}$ (*KP*) mouse model of lung adenocarcinoma, we show that Rb inactivation facilitates the bypass of two molecularly distinct barriers to tumor progression and dramatically accelerates malignant conversion and the development of metastatic disease. Although in the presence of Rb, malignant conversion requires amplification of the Raf/Mek/Erk (MAPK) signaling pathway beyond that normally activated by the Kras oncogene, we find that this requirement is abrogated when Rb is inactivated. Mechanistically, we identified Cdk2 as an important effector downstream of amplified MAPK signaling and that this activity suppresses Rb's ability to limit the adenoma-to-carcinoma transition. Importantly, inactivation of Cdk2 reduces cell proliferation in Rb wild-type cells and confers sensitivity to Cdk4/6 inhibition in both human and mouse lung adenocarcinoma cell lines that were intrinsically resistant. Acquiring metastatic competency in Rb wild-type tumors is causally linked to epigenetic changes resulting in loss of lung lineage cell fate-determining transcription factors and concomitant derepression of factors normally restricted to embryonic cell types. However, inactivation of Rb uncouples the onset of metastatic competency from the loss of lung lineage factors, facilitates the early derepression of prometastatic factors, and significantly enhances metastatic proclivity. Finally, we demonstrate that reactivation of Rb in metastatic disease settings reprograms these tumors toward a less aggressive cell state and improves overall survival. Our study highlights an unappreciated role for Rb in regulating metastasis-promoting programs, and the potential of Rb restorative therapies to treat lung adenocarcinoma. Further, we suggest a renewed investment in the development of specific Cdk2 inhibitors may be necessary for Rb pathway reactivation in certain cancer types.

IA27 Restoring Capicua (CIC) expression to limit lung cancer metastasis. R. A. Okimoto[1], Y. Lin[1], R. Ponce[1], W. Wu[1], F. Breitenbucher[2], M Schuler[2], T. Bivona[1]. [1]University of California San Francisco, San Francisco, CA, [2]West German Cancer Center, Essen, Germany.

Metastasis accounts for >90% of cancer-related death, yet the molecular effectors that promote tumor dissemination remain poorly defined. Through development of an in vivo spontaneous lung cancer metastasis model, we recently revealed that genetic inactivation of the transcriptional repressor, Capicua (CIC), through genomic deletion or loss-of-function mutations can de-repress prometastatic effectors, ETV4 and MMP24, which is necessary and sufficient for metastasis. Beyond genetic inactivation, we find that hyperactive MAPK-ERK signaling leads to functional suppression of CIC through rapid protein degradation. Collectively, these data indicate that hyperactivation of MAPK signaling may enhance metastatic potential via ERK-driven suppression of CIC that promotes ETV4-MMP24 mediated metastasis. Hyperactive ERK signaling, a hallmark of lung adenocarcinoma, can lead to rapid CIC protein degradation, which may in part explain the high rate of metastatic recurrence and poor survival in early-stage lung adenocarcinoma patients who undergo curative intent surgery. Thus, decreased CIC protein expression in the context of hyperactive ERK signaling can potentially identify a subset of patients who may benefit from more aggressive antimetastatic therapeutic strategies. To explore this, we are testing MEK-ERK blockade as a pharmacologic strategy to restore CIC protein expression, thus limiting metastatic progression by dampening the ETV4-MMP24 prometastatic axis in cancers with genetically intact CIC. Collectively, through our studies we aim to repurpose anti-MEK and anti-ERK therapeutics to restore CIC expression to block lung cancer metastasis as a prelude to clinical trials.

IA30 Investigating and overcoming primary resistance of EGFR and HER2 (ERBB2) exon 20 mutant NSCLC. J. P. Robichaux[1], Y. Y. Elamin[1], R. S. K. Vijayan[1], J. He[1], L Hu[1], F. Zhang[1], A. Poteete[1], M. Pisegna[1], M. B. Nilsson[1], H. Sun[1], M. V. Negrao[1], X. Le[1], V. M. Raymond[2], R. B. Lanman[2], G. M. Frampton[3], V. A. Miller[3], A. B. Schrock[3], J. B. Cross[1], K. Wong[4], J. V. Heymach[1]. [1]The University of Texas MD Anderson Cancer Center, Houston, TX, [2]Guardant Health, Redwood City, CA, [3]Foundation Medicine, Cambridge, MA, [4]NYU Langone, New York, NY.

EGFR and HER2 (ERBB2) exon 20 mutations occur in approximately 3.6% of NSCLC, and patients with tumors harboring these mutations have historically experienced poor response rates to clinically available TKIs. Given the poor clinical responses in these patient populations, a deeper understanding of the effect of exon 20 mutations on the drug-binding pocket, sensitivity to available TKIs, and the genomic landscape of exon 20 mutations is greatly needed. We hypothesized that while exon 20 mutations are prevalent in NSCLC, these mutations also occur in other cancer types and alter the drug-binding pocket, resulting in de novo drug resistance across cancers. To test these hypotheses, we performed an analysis of eleven databases (N=212,000) to determine the prevalence of exon 20 mutations across cancer types and utilized in silico, in vitro, and in vivo models to investigate structural alterations induced by exon 20 mutations and identify effective inhibitors. Through this analysis we found that EGFR and HER2 exon 20 mutations occur in 28 different types of cancers, and that exon 20 mutations comprise 0.6% of all cancers, amounting to approximately 16,000 patients per year in the United States. Molecular modeling and molecular dynamics simulations showed that exon 20 insertions in both EGFR and HER2 reduced the overall volume of the drug-binding pocket, which correlated with decreased sensitivity to TKIs. Through in vitro screening using more than 14 EGFR TKIs, we found that poziotinib was the most potent inhibitor tested in EGFR (N=20) and HER2 (N=6) exon 20 insertion models with IC50 values of 1.5nM and 2.5nM, respectively. In our extensive panel of Ba/F3 cells engineered to express various EGFR/HER2 mutations, poziotinib was found to be the most selective TKI for the majority of EGFR and HER2 exon 20 mutants compared to WT EGFR (Mutant/WT IC50 ratio = 0.5). In vivo, poziotinib caused 70% and 85% reduction in tumor burden in PDX models of EGFR exon 20 mutant NSCLC models harboring EGFR S768dupSVD and EGFR H773insNPH mutations after 10 days of treatment. Using genetically engineered mouse models (GEMMs) of EGFR exon 20 mutant NSCLC, poziotinib reduced tumor volume in EGFR (D770insNPG) and HER2 (Y772dupYVMA) mutant tumors by 80% and 60%, respectively, after 4 weeks of treatment. In addition, we observed that low-dose poziotinib caused an upregulation in cell surface expression of HER2 exon 20 mutants and sensitized HER2 exon 20 mutant-expressing cells to T-DM1 treatment. To exploit this, we tested the combination of low-dose poziotinib (2.5mg/kg) and a single dose of T-DM1 (10mg/kg) in an HER2 mutant NSCLC PDX model (HER2 Y772dupYVMA). We observed complete tumor regression in 20/20 mice, compared to 2/9 mice receiving T-DM1 alone or 0/12 mice receiving low-dose poziotinib by day 15 ($p<0.0001$). Median progression-free survival (mPFS, tumor doubling from best response) was 3 days, 15 days, and 27 days in vehicle control, low-dose poziotinib, and T-DM1 treated groups, whereas the mPFS had not been reached by day 45 in the combination-treated group. To validate these findings in an additional model of HER2 exon 20 mutant NSCLC, we tested low-dose poziotinib, T-DM1, and the combination in a GEMM of NSCLC harboring Y772dupYVMA. Recapitulating results seen in the PDX model, mice receiving either poziotinib or T-DM1 had on average of an 11% increase in tumor growth, whereas mice receiving the combination of low-dose poziotinib and T-DM1 had an average 47% reduction in tumor burden after four weeks. Lastly, to validate the activity of poziotinib, a phase II investigator-initiated trial (NCT03066206) testing poziotinib in patients with EGFR or HER2 exon 20 mutated NSCLC was opened. In the EGFR cohort, there was an objective response rate (ORR) of 43% and mPFS of 5.5 months in 44 evaluable patients. While the HER2 cohort is still ongoing, in the first twelve evaluable patients, there was an ORR of 42% and a mPFS of 5.6 months. Taken together, these data demonstrate that poziotinib is an effective and clinically active inhibitor for both EGFR and HER2 exon 20 mutant NSCLC and that poziotinib in combination with drug-antibody conjugates may have increased efficacy. Further, these studies demonstrate that clinical studies testing poziotinib alone and in combination with antibody-drug conjugates in other EGFR and HER2 exon 20 mutant cancers are warranted.

IA31 Genetic contributors to tumor progression and drug resistance in EGFR mutant lung cancer. K. Politi. Yale University, New Haven, CT.

Targeted therapies have transformed the landscape for the diagnosis and treatment of metastatic lung cancer. These tumors are now routinely tested for the presence of mutations or rearrangements in specific oncogenic drivers that, if present, predict sensitivity to targeted therapies directed to the genomic alterations present. Genotype-directed therapies have improved outcomes in

specific subsets of patients with metastatic lung cancer. Despite this success, targeted therapies are not curative and acquired resistance is a major impediment to cures for patients treated with these therapies. Moreover, there is heterogeneity in the durability and depth of responses between patients. A paradigm for the success of targeted therapies in lung cancer comes from Epidermal Growth Factor Receptor (EGFR) mutant lung cancer. Mutations in exons encoding the tyrosine kinase domain of EGFR confer sensitivity to tyrosine kinase inhibitors (TKIs), and several are currently approved for the first-line treatment of EGFR mutant lung cancer. Most recently, the third-generation TKI osimertinib was approved and is increasingly being used in the first line. However, we have very limited knowledge of the mechanisms of resistance to osimertinib given its recent adoption in the clinic. Without knowledge about resistance mechanisms, optimal post-osimertinib treatment strategies remain to be defined. We modeled acquired resistance to first-line osimertinib treatment in transgenic mouse models of EGFRL858R-induced lung adenocarcinoma and found that it is mediated largely through secondary mutations in EGFR and identified therapeutic strategies to treat these tumors and prevent their emergence. Moreover, since EGFR mutant tumors in patients harbor additional genetic alterations beyond EGFR, many of them in tumor suppressor genes, we tested how the presence of co-occurring genetic alterations in tumor suppressor genes contributes to the progression and osimertinib sensitivity of the tumors in the mouse models of EGFR mutant lung cancer. Collectively, our findings highlight how genetically engineered mouse models of lung cancer, including those with complex genotypes, can be leveraged to study tumor progression and drug resistance in vivo.

IA33 Mechanisms of small-cell lineage transformation in resistance to targeted therapies. Y. Inoue, W. Lockwood. BC Cancer, Vancouver, BC, Canada.

EGFR tyrosine kinase inhibitors (TKIs) are highly effective for tumors with EGFR mutations. However, resistance to these compounds remains a major issue, with the most frequent mechanism including the acquisition of a secondary mutation in EGFR (T790M) (1), followed by amplification of the hepatocyte growth factor receptor (MET) gene (2) and mutations in BRAF and PIK3CA genes (3,4). Epithelial–mesenchymal transition (EMT) and lineage transformation are less frequent but also prevalent, with up to 15% of cases with acquired resistance to first- and second-generation EGFR TKIs demonstrating histologic change from lung adenocarcinoma (LUAD) to small-cell lung cancer (SCLC) (4). Histologic plasticity as a mechanism of resistance is becoming increasingly prominent as other resistant mechanisms can now be successfully targeted (5). Currently, as with de novo SCLC, conventional platinum doublet chemotherapy is the standard of care for patients with treatment-induced SCLC. Unfortunately, this treatment often produces an incomplete and nondurable response followed by inevitable relapse within months, leading to poor patient outcomes (6). Thus, this mechanism of resistance will represent a major barrier towards the success of third-generation TKIs, and new strategies to prevent this lineage shift or to treat SCLC transformed tumors are urgently needed. Despite the increasing clinical importance, the biologic pathways regulating LUAD to SCLC transformation are poorly understood. Assessment of clinical samples has revealed that EGFR-mutant tumors universally lose EGFR protein expression upon SCLC transformation, despite still harboring EGFR mutation (7). Furthermore, the mutation spectrum of these transformed cases includes inactivation of the tumor suppressors RB and p53 in nearly all cases, mirroring de novo SCLC (7). However, accumulating experimental evidence has demonstrated that while necessary, dual inactivation of RB and p53 is not sufficient to cause SCLC lineage transformation in EGFR-mutated LUAD, suggesting that additional factors are required (7). MYC amplification and PIK3CA mutation have been proposed to potentially cooperate with RB/p53 loss to facilitate transformation (8), and specific epigenetic regulators may also provide the appropriate context for lineage reprogramming to occur. Despite this, no in vitro or in vivo models of SCLC transformation in EGFR TKI resistance have been developed, making it difficult to comprehensively explore the molecular events driving this lineage shift. Interestingly, there are clear differences between LUAD and SCLC regarding EGFR expression and gene alterations in MAPK pathway including EGFR/KRAS mutations: EGFR is usually not expressed (9) and EGFR/KRAS mutations are extremely rare in SCLC (10); in contrast, EGFR/KRAS play crucial roles in LUAD biology, including regulating differentiation in addition to proliferation (11). To date, however, no clear explanation has been given for these differences. We have recently shown that activation of MAPK signaling in SCLC leads to suppression of the neuroendocrine phenotype—including downregulation of the transcription factors NEUROD1, INSM1, BRN2, and ASCL1—and transformation to an NSCLC-like state (12). Using this model system, we have begun to elucidate the key transcription factors and epigenetic changes that drive SCLC to NSCLC transformation in the hope that the same processes will also be involved in the clinically relevant scenario: SCLC transformation

from EGFR mutant LUAD during TKI resistance. We suggest that only EGFR-mutant LUADs that do not reactivate MAPK signaling through secondary EGFR mutations or alterations in parallel kinase pathways (i.e., MET) during development of TKI resistance will be able to undergo SCLC lineage transformation, and that RB/p53 loss and epigenetic plasticity provide the permissive context in which this transformation can occur. Greater understanding of lineage transformation in LUAD will provide important insights in terms of managing outcomes of patients undergoing targeted therapy and offer new avenues towards treatment of TKI resistant tumors.

References: 1. Kobayashi S et al. NEJM 2005;352:786-92. 2. Bean J et al. PNAS 2007;104:20932-7. 3. Ohashi K et al. PNAS 2012;109:E2127-33. 4. Sequist LV et al. Sci Transl Med 2011;3:75ra26. 5. Mok TS et al. NEJM 2017;376:629-40. 6. Roca E et al. Cancer Treat Rev 2017;59:117-22. 7. Niederst MJ et al. Nat Commun 2015;6:6377. 8. Lee JK et al. J Clin Oncol. 2017;35:3065-74. 9. Gamou S et al. Cancer Res 1987;47:2668-73. 10. Cristea S et al. J Thorac Oncol 2016;11:1233-41. 11. Byers LA et al. Cancer Discov 2012;2:798-811. 12. Inoue Y, Lockwood W. J Thorac Oncol 2018;13:S433–34.

IA34 The YAP/FOXM1 axis regulates EMT-associated EGFR tyrosine kinase inhibitor resistance and increased expression of spindle assembly checkpoint components. M. B. Nilsson[1], H. Sun[1], J. Robichaux[1], L. Diao[2], Y. Xi[2], P. Tong[2], L. Sheng[2], M. Hofstad[1], M. Kawakami[1], X. Le[1], X. Liu[1], Y. Fang[1], A. Poteete[1], M. Vailati Negrao[1], H. Tran[1], E. Dmitrovsky[1], D. Peng[1], D. Gibbons[1], J. Wang[2], J. V. Heymach[1]. [1]Thoracic/Head and Neck Medical Oncology, The University of Texas MD Anderson Cancer Center, Houston, TX, [2]Bioinformatics and Computational Biology, The University of Texas MD Anderson Cancer Center, Houston, TX.

While EGFR mutant NSCLC patients are initially responsive to EGFR targeted therapies, resistant disease inevitably emerges. In nearly half of resistance cases, tumors lack secondary EGFR mutations such as T790M and are refractory to 2nd- and 3rd-generation EGFR tyrosine kinase inhibitors (TKI). We and others have also observed that EGFR-independent resistant tumor cells may undergo a histologic and functional transformation through epithelial-to-mesenchymal transition (EMT) (Byers et al., 2013; Chung et al., 2011; Uramoto et al., 2010; Zhang et al., 2012), which can occur concurrently with other genomic alterations. The lack of treatment regimens with efficacy against EGFR-independent EGFR TKI resistance remains a major clinical challenge. We investigated transcriptomic and proteomic alterations that occur in NSCLC cells with acquired resistance to EGFR TKIs that occurs independent of EGFR and c-Met and screened >1,300 compounds to identify targetable vulnerabilities. T790M-negative EGFR TKI resistance was associated with evidence of a mesenchymal transition along with increased activation of the YAP/FOXM1 transcriptional program and a broad-spectrum multidrug resistance phenotype. EGFR TKI resistant cells displayed increased expression of spindle assembly checkpoint (SAC) proteins PLK1, Aurora kinases, survivin, and KSP, and expression of these proteins was dependent on the YAP/FOXM1 axis. Consistent with recent reports (Bertran-Alamillo et al., 2019; Shah et al., 2019), EGFR TKI resistant cells were found to be sensitive to aurora kinase inhibitors. We further determined that EGFR TKI resistant cells were likewise highly sensitive to inhibitors of components of the spindle assembly checkpoint (SAC) pathway including PLK1, KSP, and survivin, and treatment with these agents resulted in the accumulation of cells in the G2/M phase of the cell cycle and mitotic catastrophe. Using a patient-derived model of T790M negative EGFR TKI resistance, we observed that treatment with SAC component inhibitors, alisertib, ispinesib, or volasertib significantly inhibited tumor growth compared with vehicle-treated tumors. Analysis of NSCLC clinical data revealed that FOXM1 expression correlated with expression of SAC components including PLK1, Aurora kinases, KSP, and survivin. Moreover, in EGFR mutant NSCLC patients, high FOXM1 expression was associated with a worse clinical outcome compared to EGFR mutant NSCLC patients with low expression of FOXM1. In resistant models, targeting of YAP reduced FOXM1 expression and expression of SAC components. In conclusion, we provide novel insights into the molecular alterations associated with EGFR TKI resistance and demonstrate that upregulation of SAC components in EGFR TKI resistant cells occurs through the activation of the YAP/FOXM1 pathway. These results support the future targeting of these pathways in NSCLC patients with EGFR-independent resistance to EGFR-targeted agents.

PR01 Transcriptional subtypes resolve tumor heterogeneity and identify therapeutic vulnerabilities in lung cancer. A. Daemen[1], J. E. Cooper[2], S. Myrta[3], M. Wongchenko[2], E. Lin[2], J. E Long[2], O. Foreman[2], Z. Modrusan[2], J. Tremayne[2], C. C. de la Cruz[2], M. Merchant[2], S. E Martin[2], Y. Yan[2], M. R Junttila[1]. [1]ORIC Pharmaceuticals, South San Francisco, CA, [2]Genentech, Inc., South San Francisco, CA, [3]Roche, Warsaw, Poland.

Non-small cell lung cancer (NSCLC) is the leading cause of cancer death. Adenocarcinomas are the most prominent type of NSCLC, characterized by intense diversity both with respect to genetics and therapeutic response. More than 75% of adenocarcinomas are devoid of therapeutic options due to lack of druggable driver events. Herein, we deconvolute tumor heterogeneity in order to discover therapeutically tractable dependencies in this patient subset. By relying on transcriptome and genetic data from >800 patient tumors from the early and advanced setting, we identify three stable and reproducible tumor subtypes. In patients, the subtypes are not strongly associated with genetic events and multiregional biopsies demonstrated subtype stability, despite genetic diversity. We also identified context-dependent prognostic relevance for the transcriptional subtypes. Further interrogation revealed that genetically engineered murine models (GEMM) recapitulate human lung adenocarcinoma subtypes and can therefore be used to discover subtype-specific dependencies. We identified significant differences in subtype-selective sensitivity to MEK inhibitors using an unbiased chemical screen, which reproduced across model systems and validated in a clinical trial. Our results shed new light on MAPK dependence and provide proof of concept for the therapeutic relevance of the transcriptional subtypes, the fidelity of various in vitro and in vivo model systems, and demonstrate that these preclinical findings can be confirmed in a clinical trial. Further exploring subtype dependencies has the potential to improve targeting of lung adenocarcinoma tumors.

This abstract is also being presented as Poster A09.

PR02 The SHP2 inhibitor RMC-4630 in patients with KRAS-mutant non-small cell lung cancer: Preliminary evaluation of a first-in-man phase 1 clinical trial. S. I. Ou[1], M. Koczywas[2], S. Ulahannan[3], P. Janne[4], J. Pacheco[5], H. Burris[6], C. McCoach[7], J. S. Wang[8], M. Gordon[9], E. Haura[10], J. W. Riess[11], V. Zhu[1], K. Ng[4], S. G. Eckhardt[12], A. Capasso[12], R. Dua[13], A. Chen[13], Z. Wang[13], J. Hayes[13], R. Nichols[13], T. Bivona[7]. [1]University of California Irvine, Irvine, CA, [2]City of Hope Hospital, Duarte, CA, [3]University of Oklahoma, Norman, OK, [4]Dana-Farber Cancer Institute, Boston, MA, [5]University of Colorado Denver, Denver, CO, [6]Sarah Cannon Research Institute, Nashville, TN, [7]University of California San Francisco, San Francisco, CA, [8]Florida Cancer Specialists, Fort Myers, FL, [9]Honor Health, Scottsdale, AZ, [10]Moffitt Cancer Center, Tampa, FL, [11]University of California Davis, Davis, CA, [12]University of Texas Austin, Austin, TX, [13]Revolution Medicines, Redwood City, CA.

RMC-4630 is a potent, selective, orally bioavailable allosteric inhibitor of SHP2, a central node in receptor tyrosine kinase and RAS signaling cascades. Preclinical data have demonstrated that RMC-4630 can inhibit growth and induce regressions in tumors carrying certain driver mutations in the RAS signaling pathway that are "semi-autonomous," such as $KRAS^{G12C}$, $NF1^{LOF}$, and $BRAF^{Class3}$. A phase 1 dose-escalation trial of RMC-4630 is currently testing a daily dosing schedule and an intermittent dosing schedule. A total of 56 patients have been dosed, of whom 23 had NSCLC (19/23 with KRAS mutations). For patients with NSCLC harboring a $KRAS^{G12C}$ mutation, the disease control rate (DCR) was 5/7 (71%) with reduction in tumor volume reported in three patients (43%). Preliminary clinical antitumor activity was also seen in one additional patient with NSCLC harboring the oncogenic $KRAS^{G12D}$ mutation and a presumed hyperactivating SHP2 mutation ($SHP2^{V428M}$). Plasma exposures of RMC-4630 increased proportional to dose, and at all dose levels were within the range that was projected to have antitumor activity from preclinical studies. Sequential analysis of pERK in peripheral blood cells and paired tumor biopsies showed evidence of RAS signaling pathway inhibition. The safety and tolerability profile of RMC-4630 appear to be consistent with RAS pathway inhibition. RMC-4630 showed reasonable tolerability and preliminary signs of clinical activity in patients with NSCLC harboring KRAS mutations. RMC-4630 continues to be tested as a single agent in patients with tumors harboring RAS signaling pathway mutations. This study is also open to patients with $KRAS^{G12C}$ NSCLC who are progressing on $KRAS^{G12C}$(OFF) inhibitors. A study in combination with the MEK inhibitor cobimetinib (Cotellic) is also under way. RMC-4630, and other chemically related SHP2 inhibitors, have demonstrated combinatorial benefit with mutant-selective inhibitors of $KRAS^{G12C}$(OFF), such as AMG 510, in preclinical models. A clinical trial evaluating the combination of RMC-4630 and AMG 510, as well as additional combination studies, are planned.

This abstract is also being presented as Poster A12.

PR03 The genome-wide mutational landscape of lung cancer in never-smokers: The Women's Health Initiative (WHI) cohort. S. Moorthi, A. Paguirigan, G. Anderson, P. Porter, M. Holden, G. Ha, A. H. Berger. Fred Hutchinson Cancer Research Center, Seattle, WA.

Lung cancer is the leading cause of cancer deaths worldwide, and a positive history of smoking remains one of the most significant risk factors for lung cancer development. However, about 20% of lung cancer diagnoses are reported in individuals with no smoking history. Lung cancer in never-smokers (LCNS) is clinically distinct from tobacco-induced lung cancer, with a greater proportion of LCNS occurring in women and having adenocarcinoma histology. One of the key challenges in identifying the cancer-promoting genetic events that drive LCNS is the relatively small number of tumors that have been sequenced using genome- or exome-wide approaches. We address this gap through the collection and exome sequencing of lung tumor tissue and matched blood-derived normal DNA from 77 women who participated in the Women's Health Initiative (WHI), the majority of whom have light or no smoking history. Samples were sequenced with a custom exome approach at the Center for Cancer Genome Discovery (CCGD), Dana-Farber Cancer Institute, with baits for all protein coding regions of the genome and noncoding regions that are frequently rearranged/translocated in lung cancer, such as introns within the *ALK* gene. Somatic mutations were identified using MuTect2 and mutational significance was determined using MutSig2CV. Preliminary analysis involving 18 tumor/normal pairs identified an enrichment of somatic alterations in genes such as *EGFR* and *TP53*. Additionally, tumor purity and copy number alterations were estimated using ichorCNA. Translocation analysis was performed using BreaKmer identifying a CD74-ROS1 fusion. 72% of the cases harbored previously known oncogenic drivers of lung adenocarcinoma such as mutations in *EGFR*, *KRAS*, *RIT1*, and *MET* with mutations that are clinically targetable using FDA-approved or investigational agents. Overall this project will double the number of exome profiles from never-smokers and, importantly, leverage the extensive metadata curated under the WHI to evaluate secondary/environmental factors such as second-hand smoke and radon exposure and their potential role in LCNS.

This abstract is also being presented as Poster A24.

PR04 Integrated proteometabolomic analysis reveals metabolic vulnerabilities in small-cell lung cancer. A. Prabhu[1], K. Scott[1], P. Stewart[1], D. Grass[1], M. Fernandez[1], J. Koomen[1], T. Bannister[2], S. Sumner[3], C. Rudin[4], G. Denicola[1], J. Cleveland[1], E. Haura[1]. [1]H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL, [2]The Scripps Research Institute, Jupiter, FL, [3]University of North Carolina, Chapel Hill, NC, [4]Memorial Sloan Kettering Cancer Center, New York, NY.

Small-cell lung cancer (SCLC) is the third most common histology of lung cancers and is extremely aggressive and highly metastatic. Although SCLC responds well to radiation and standard platinum-based chemotherapy, this is nearly invariably followed by relapse and the emergence of chemoresistant disease. Hence, SCLC has been declared a recalcitrant malignancy by the NCI, and there is an urgent need to identify new and actionable therapeutic vulnerabilities for treatment-naïve and chemoresistant SCLC. To this end we performed unbiased activity-based (ATP-binding) proteome profiling (ABPP), expression proteomics, and untargeted metabolomics on a panel of SCLC and NSCLC cell lines, patient-derived lung tumor tissues, and PDX including paired treatment-naïve and cisplatin-resistant SCLC. These studies revealed highly elevated activity of enzymes associated with glycolysis, lipid biosynthesis, and purine metabolism in SCLC. In addition, metabolomic analysis identified concordant upregulation of metabolites in these pathways in SCLC. We further performed screening with available metabolic drugs on SCLC and NSCLC cell lines. The results showed that the MCT1/MCT2 lactate transport inhibitor SR-13800 and the PFKFB3 inhibitors 3PO and PFK15 compromised SCLC cell growth and their combined inhibition showed synergy, provoking rapid SCLC cell death. Flux, metabolomic, and genetic (CRISPR-editing) analysis of SCLC cells revealed that MCT1/2 inhibition loss blocked glycolysis and provoked a shift towards oxidative phosphorylation (OXPHOS), and that this provoked increases in intracellular lactate and dihydroxyacetone phosphate (DHAP) and a marked shift in the NAD+ to NADH ratio towards NADH. In addition, levels of amino acids that can generate NAD+ were also significantly reduced. In contrast and surprisingly, PFKFB3 inhibition led to a collapse in OXPHOS and provoked increases in glycolysis and increased efflux of lactate. The combined inhibition of MCT1/2 and PFKFB3 amplified the metabolic deficits provoked by MCT1/2 and led to metabolic collapse via suppression of both glycolysis and OXPHOS. Thus, cotargeting MCT1/2 and PFKFB3 provokes synthetic lethality in SCLC, supporting the notion that their dual inhibition will be an effective treatment strategy for this lethal malignancy.

This abstract is also being presented as Poster A22.

PR05 A reservoir of tumor-specific CD8 T cells in lung cancer resides in the draining lymph node. K. Connolly, B. Fitzgerald, M. Nader, N. Joshi. Yale University, New Haven, CT.

Recent work has described the population of CD8 T cells that respond to anti-PD-1 therapy (marked by TCF1 and PD-1), but it remains unclear how these T cells are maintained within the immunosuppressive tumor microenvironment (TME) of lung cancer. To understand this, we developed a genetically engineered model in which Kras-G12D expressing p53 deficient lung adenocarcinomas express a known neoantigen called the iNversion INduced Joined neoAntigen (NINJA). NINJA allows us to follow neoantigen-specific CD8 T cells over the course of tumor development. We find that ~20% of tumor-specific T cells in early 8-wk tumors are TCF1+, but by 17-20 wks, this TCF1+ has significantly shrunk, and there has been a concomitant increase in the expression of markers of T-cell terminal differentiation (Tim3). This is consistent with the idea that T cells receive signals in the TME that drive terminal differentiation and restrict responses to immunotherapy. We reasoned that if the signals driving terminal differentiation were provided in the TME, neoantigen-specific T cells in the tumor-draining lymph node (dLN) may remain less differentiated over the course of tumor development. Analysis of tumor-specific T cells in the dLNs of 8-wk and 17-wk tumors showed that they were mostly TCF1+. Moreover, single-cell transcriptional analyses suggested that these cells were less differentiated than their counterparts in tumors. T-cell receptor (TCR) signals are a major driver of terminal differentiation, and we observed that tumor-specific T cells in the dLN were not receiving TCR signals, while all T cells in the TME were positive for TCR signals. This suggested at least two models for how antitumor T cells function: 1) tumor-reactive T cells in dLNs and TME could function independently of one another, or 2) tumor-reactive T cells might have a role in sustaining the antitumor T-cell response over the course of lung-tumor development through migration. Consistent with the latter model, TCR sequencing of dLN and TME neoantigen-specific T cells showed a close clonal relationship: 13 of the top 15 clones in the TME were present in the dLN, and the hierarchy of clonal dominance was maintained. This was also true in 17-wk tumor-bearing mice, suggesting that the population of tumor-specific CD8 T cells in the dLN serves as a reservoir of less differentiated cells that can continuously replenish the T cells in the TME, helping to sustain the antitumor T-cell response over the course of tumor development. Critically, it is unclear whether current immunotherapeutic strategies leverage this reservoir of T cells, raising the possibility that this population of dLN tumor-specific T cells could be targeted to provide significant additional benefit for patients receiving immunotherapy.

This abstract is also being presented as Poster A31.

PR06 Blockade of myeloid suppressor cells overcomes the anti-PD-1/PD-L1 resistance in KRAS-driven and LKB1-deficient NSCLC. R. Li[1], R Salehi-Rad[1], M. Momcilovic[1], R. Lim[1], S. Ong[1], Z. Huang[1], L. Tran[1], J. Zhe[1], M. Paul[1], M. Teitell[1], J. Minna[2], K. Krysan[1], D. Shackelford[1], B. Liu[1], S. Dubinett[1]. [1]University of California Los Angeles, Los Angeles, CA, [2]UTSW, Dallas, TX.

KRAS mutations account for approximately 30% of non-small cell lung cancer (NSCLC). Targeted therapies against KRAS mutations are still lacking. Although treatment with checkpoint inhibitors (IOs) can achieve a durable antitumor response in lung cancer patients, including those harboring KRAS mutations, the clinical benefit varies. Patients harboring KRAS/LKB1 comutation, which occurs in 30% of KRAS-mutant NSCLC, have a significantly lower response rate to IOs compared to those with KRAS mutations alone or KRAS/TP53 comutation. However, the mechanisms of this resistance are not well elucidated. In this study, we showed that LKB1 deficiency activated the MARKs-dependent NF-κB pathway and resulted in increased secretion of CXCR2 ligands, including CXCL1, CXCL2, CXCL3, CXCL5, and CXCL8, in human bronchial epithelial cell lines, NSCLC cell lines, and patient-derived xenografts. Elevation of these CXCR2 ligands was also observed in the KrasK12D;Lkb1-/- (KL) tumors from a genetically engineered mouse model and in the KrasK12D;Tp53+/-;Lkb1-/- (KPL) tumors from a syngeneic mouse model, compared to their KrasK12D;Tp53-/- (KP) counterparts. The immune phenotype of these KL or KPL tumors demonstrated a significantly higher percentage of polymorphonuclear myeloid-derived suppressor cells (PMN-MDSCs) in the tumor microenvironment (TME), consistent with the known function of CXCR2 ligands. Utilizing the syngeneic murine lung cancer model, we revealed that KPL tumors with a high mutational load had a significantly lower anti-PD-1 response compared to the KP tumors. Therefore, we hypothesized that PMN-MDSCs may cause resistance to anti-PD-1 monotherapy in LKB1-deficient tumors. We found that an anti-PD-1 antibody combined with MDSC depletion via an anti-Gr-1 antibody or induction of MDSC differentiation via retinoid acid could lead to complete tumor eradication. Rechallenge with the same KPL tumor cells in cured

mice, three months after the initial treatment, resulted in a rapid tumor rejection, suggesting a durable systemic antitumor immune response. We found increased tumor-infiltrating antigen-presenting cells, CD8+ T cells, and NK cells, and decreased T regulatory cells in the TME following the combination therapy. In conclusion, we have revealed increased CXCR2 ligand production and tumor-infiltrating PMN-MDSCs in NSCLC with KRAS and LKB1 comutation. MDSC blockade potentiates the efficacy of anti-PD-1 therapy in these tumors.

This abstract is also being presented as Poster A11.

PR07 Dendritic cell in situ vaccination potentiates anti-PD-1 efficacy and induces immunoediting in a murine model of NSCLC. R. Salehi-Rad, R. Li, R. Lim, L. Tran, J. Abascal, S. Ong, B. Liu, S. Dubinett. University of California Los Angeles, Los Angeles, CA.

Studies reveal that responses to checkpoint blockade in non-small cell lung cancer (NSCLC) are associated with high tumor mutational burden (TMB), preexisting $CD8^+$ T-cell infiltration, and high baseline PD-L1 expression within the tumor microenvironment (TME). In contrast, co-occurring KRAS/LKB1 mutation is associated with primary resistance to PD-1 blockade and decreased overall survival. In preclinical studies as well as a phase I clinical trial, we have discovered that intratumoral (IT) vaccination with gene-modified dendritic cells expressing CCL21 (CCL21-DC) promotes tumor effector T-lymphocyte infiltration, PD-L1 upregulation, and systemic tumor-specific immune responses. We hypothesized that in situ vaccination with CCL21-DC could restore tumor antigen presentation and promote T-cell priming and activation, thereby sensitizing nonresponsive NSCLC tumors to checkpoint blockade. Although genetically engineered murine models (GEMMs) of NSCLC bear driver mutations of the disease, recent studies reveal that these GEMMs possess low mutational burden. We established novel GEMMs of NSCLC [$Kras^{G12D}$ (K), $Kras^{G12D}P53^{-/-}$ (KP), $Kras^{G12D}P53^{+/-}Lkb1^{-/-}$ (KPL)] bearing common driver mutations and varying mutational loads by in vitro exposure of tumor cell lines to the carcinogen N-methyl-N-nitrosourea (MNU). Our preclinical KPL model with high TMB recapitulates the immunologic phenotype of human disease and contains a predominance of myeloid-derived suppressor cells (MDSC), low tumor-infiltrating lymphocytes (TILs), and low PD-L1 expression within the TME. As anticipated, the KPL tumors are resistant to anti-PD-1 therapy, even with increased mutational load. We evaluated IT CCL21-DC combined with anti-PD-1 therapy in immunocompetent mice bearing KPL tumors with high TMB and observed that IT CCL21-DC vaccination induces infiltration of autologous T lymphocytes and conventional type I DCs (cDC1s) into the TME and sensitizes the tumors to anti-PD-1 therapy. Combination therapy also reprogrammed the myeloid compartment, resulting in a significant reduction of MDSCs and a concurrent increase in $CD11b^+Ly6G^{hi}Ly6C^{lo}$ monocyte/myeloid population. Whole-exome sequencing (WES) of tumors revealed immunoediting and selective depletion of tumor subclones post IT CCL21-DC and anti-PD1 combination therapy. Future studies will evaluate the evolution of the T-cell receptor (TCR) repertoire in response to the combination treatment and define functional responses to neoepitopes. These studies will enhance our understanding of the molecular mechanisms of tumor vaccination and facilitate the development of rational combination strategies.

This abstract is also being presented as Poster A35.

PR08 Unraveling the mechanisms of small-cell lung cancer brain metastasis. F. Qu[1], A. Pasca[1], C. Kong[2], M. Winslow[3], J. Sage[4]. [1]Department of Pediatrics, Stanford University School of Medicine, Stanford, CA, [2]Department of Pathology, Stanford University School of Medicine, Stanford, CA, [3]Department of Genetics, Department of Pathology, Stanford University School of Medicine, Stanford, CA, [4]Department of Pediatrics, Department of Genetics, Stanford University School of Medicine, Stanford, CA.

Brain metastases are the most common type of intracranial tumors and are associated with high morbidity and mortality rates in cancer patients worldwide. Therapeutic options to treat brain metastases remain extremely limited, in part because of a lack of preclinical models and a limited understanding of the mechanisms allowing tumor cells from various primary sites to grow in the brain microenvironment. Small-cell lung cancer (SCLC) is a highly lethal type of lung cancer that frequently metastasizes to the brain. We have recently developed two preclinical models to investigate the interactions of SCLC cells with cells in the brain microenvironment and to identify mechanisms of SCLC brain metastasis. First, we have developed a direct intracranial transplant approach in which fluorescently labeled SCLC cells form tumors in the brain of recipient mice, including immunocompetent mice. Second, we have developed a coculture system in which SCLC cells invade brain organoids engineered from human iPS cells. Using these two models, we have found that GFAP-positive reactive astrocytes interact with SCLC cells in

the brain and potentially affect the growth of SCLC brain metastases. GFAP-positive astrocytes actively infiltrate SCLC brain metastases in our preclinical models and in patients. We also show that astrocytes can promote the growth of SCLC cells in culture. Previous studies have shown that Nfib, an oncogenic transcription factor that drives the metastatic progression of SCLC, can induce expression of neuronal gene programs in metastatic SCLC cells. Neuron-astrocyte interactions play a critical role in neuronal growth and migration during development. Therefore, in an effort to determine the mechanisms underlying the interactions between SCLC cells and astrocytes, we have knocked down Nfib in SCLC cells and transplanted them into mouse brains. We found that Nfib is critical for the growth of SCLC brain metastases and that SCLC tumors with reduced Nfib expression show elevated rates of apoptosis, impaired invasion, and decreased astrocyte infiltration. Ongoing work is focusing on characterizing Nfib-downstream factors that are critical for SCLC growth and migration in the brain microenvironment, especially those with functions in neuron-astrocyte interactions. These studies will provide better mechanistic insight into how cancer cells adapt to and grow in the brain microenvironment, which may eventually help identify new therapeutic targets to treat brain metastases.

This abstract is also being presented as Poster B23.

PR09 IHH acts as a tumor suppressor of lung adenocarcinoma by repressing reactive oxygen species. Sahba Kasiri[1], Baozhi Chen[1], Alexandra Wilson[1], Annika Reczek[1], Simbarashe Mazambani[2], Jashkaran Gadhvi[2], Evan Noel[1], Ummay Marriam[1], Barbara Mino[3], Wei Lu[3], Luc Girard[1], Luisa Solis[3], Katherine Luby-Phelps[1], Justin Bishop[1], Jung-whan Kim[2], James Kim[1]. [1]UT Southwestern Medical Center, Dallas, TX, [2]The University of Texas at Dallas, Dallas, TX, [3]The University of Texas MD Anderson Cancer Center, Houston, TX.

Background: Aberrant activation of the Hedgehog (Hh) signaling pathway, a crucial developmental pathway, drives the tumor growth of basal cell carcinoma, medulloblastoma, and rhabdomyoma. However, recent data suggest that paracrine activation of the pathway is tumor suppressive rather than oncogenic in sporadic epithelial cancers. The role of the pathway in non-small lung cancer is poorly understood. Thus, we explored the role of stromal Hh pathway activation in growth of lung tumor epithelia.

Methods: Human and murine lung adenocarcinoma cell lines and murine fibroblasts were used to probe SHH mRNA and protein expression and to verify paracrine activation of the Hh signaling pathway. The role of paracrine SHH was tested in vivo using $Kras^{LSL\text{-}G12D/+}$;$Trp53^{fl/fl}$ (KP) and LSL-$Kras^{G12D/+}$;$Trp53^{fl/fl}$;$Shh^{fl/fl}$ (KPS) autochthonous murine lung cancer models. The role of IHH was examined in vivo using the pSECC CRISPR system in KP;$Rosa26^{LSL\text{-}fLuc/+}$ and tumor growth monitored by bioluminescence imaging.

Results: In human lung adenocarcinoma (LAD) patients, higher expression of *SHH* mRNA correlated with poor overall and progression free survival. Coculture of high SHH-expressing tumor epithelial cells and Shh-Light2 reporter fibroblasts demonstrated that SHH activated the Hh pathway in the fibroblasts in a paracrine manner. Surprisingly, genetic loss of SHH in an autochthonous mouse model, KPS, did not affect overall survival compared to KP mice. However, early inhibition of stromal Hh pathway by 5E1, an anti-SHH/IHH antibody, in KP mice resulted in significantly worse survival with increased metastatic burden. We tested the loss of IHH in vivo with the pSECC CRISPR system. IHH-loss in airway epithelia led to more aggressive tumor growth, suggesting that IHH, not SHH, activates the pathway in stroma to drive its tumor suppressive effects--a novel role for IHH in the lung. Tumors from mice treated with 5E1 had decreased blood vessel density and increased reactive oxygen species (ROS). Treatment of KP mice with 5E1 and N-acetylcysteine, as a ROS scavenger, decreased tumor ROS levels, inhibited tumor growth, and prolonged mouse survival, suggesting that increased ROS levels from stromal Hh pathway inhibition accelerated lung tumor growth.

Conclusions: IHH activates the Hh signaling pathway in lung stroma in a paracrine manner to suppress tumor growth and metastases, in part, by limiting ROS production.

This abstract is also being presented as Poster B27.

PR10 Targeting glucose reliance in lung squamous cell carcinoma. M. Hsieh[1], S. Mazambani[2], J. Kim[2]. [1]UTSW, Dallas, TX, [2]UT Dallas, Richardson, TX.

Lung squamous cell carcinoma (LSCC) is a major class of pulmonary malignancy that accounts for 25-30% of all lung cancers. LSCC patients have benefited very little from the application of targeted therapeutic options. As a result, decades-old platinum-based chemotherapy or radiation regimens with limited efficacy and specificity remain the first-line treatment options. Therefore, identification and elucidation of

targetable vulnerabilities in LSCC is urgently needed to improve therapeutic outcomes in LSCC patients. Our efforts to identify targetable pathways crucial for LSCC growth and survival led to the discovery of exceptional overexpression of glucose transporter 1 (GLUT1, encoded by the SLC2A1 gene) and exceptional glucose reliance for tumor growth and survival. Mechanistically, our recently published study demonstrated that squamous lineage transcription factors, p63 and SOX2, jointly transactivate an intronic enhancer cluster in the SCL2A1 gene, and this hyperactive GLUT1-mediated glucose influx provides a carbon source to enhance the antioxidative capacity and tumorigenecity of LSCC. This previously unrecognized metabolic signature phenotypically embedded in the squamous lineage subtype of lung cancer provides a rationale to target GLUT1-mediated glucose influx. We evaluate the efficacy of ketogenic diet (dietary glucose restriction) as well as the SGLT2 inhibitor canagliflozin, an FDA-approved drug for the treatment of type 2 diabetes (pharmacologic glucose restriction), which effectively lowers the host blood glucose levels by blocking SGLT2-mediated renal glucose reabsorption. Reduction of blood glucose lowers blood insulin levels, which effectively suppresses PI3K/AKT signaling in LSCC cells. Repurposing FDA-approved canagliflozin can be rapidly translatable as an effective therapeutic strategy for squamous cancer patients.

This abstract is also being presented as Poster A21.

PR11 Proteogenomic characterization reveals therapeutic vulnerabilities in lung adenocarcinoma. M. A. Gillette[1], S. Satpathy[1], S. Cao[2], S. Dhanasekaran[3], S. Vasaikar[4], K. Krug[1], F. Petralia[5], Y. Li[2], W.-W. Liang[2], B. Reva[5], R. Hong[6], S. Savage[7], G. Getz[1], Q. K. Li[8], B. Zhang[7], H. Rodriguez[9], K. Ruggles[6], A. I. Robles[9], K. C. Clauser[1], R. Govindan[2], P. Wang[5], A. Nesvizhskii[3], L. Ding[2], D. R. Mani[1], S. A. Carr[1]. [1]Broad Institute of MIT and Harvard, Cambridge, MA, [2]Washington University in St. Louis, St. Louis, MO, [3]University of Michigan, Ann Arbor, MI, [4]University of Texas MD Anderson Cancer Center, Houston, TX, [5]Icahn School of Medicine at Mt. Sinai, New York, NY, [6]NYU School of Medicine, New York, NY, [7]Baylor College of Medicine, Houston, TX, [8]Johns Hopkins University School of Medicine, Baltimore, MD, [9]National Cancer Institute, Bethesda, MD.

A persistent central deficiency in our knowledge of cancer concerns how genomic changes drive the proteome and phosphoproteome to execute phenotypic characteristics. Furthermore, increasing evidence implicating epigenetic and post-translational changes in cancer biology reinforce the notion that molecular profiles based on nucleic acids are incomplete and are critically complemented by analyses of proteins and their post-translational modifications (PTMs). We present the first integrated proteogenomic study on a prospectively collected lung adenocarcinoma (LUAD) cohort, and provide new insights including on molecular taxonomy, novel mutations and fusions, protein and PTM associations with canonical driver mutations, metabolic dependencies and the immune milieu. The National Cancer Institute's Clinical Proteomics Tumor Analysis Consortium (CPTAC) collected 110 LUAD tumors and 101 paired normal adjacent tissues using rigorous standard protocols to minimize ischemic time and other pre-analytical variability. Approximately equal numbers of Eastern (China, Vietnam) and Western patients were enrolled and the population included ~ 40% never-smokers. Comprehensive genomic and proteomic characterization provided whole exome, whole genome, copy number, RNAseq, microRNA, long non-coding RNA, methylation, global proteome, phosphoproteome, and acetylome data. The distribution of top driver mutations paralleled that of large genomics studies; both novel structural variants in established driver genes and novel ALK fusion partners were defined. 120 proteins including CLDN18, ANK1 and PTPRCAP had evidence of regulation by DNA methylation. Association analyses highlighted important outliers seen only in the phosphoproteomic data, including potential therapeutic targets such as SOS1 in KRAS mutant and PTPN11 (Shp2) in EGFR mutant tumors. Novel KEAP1 mutants were described including one suggesting an alternative mechanism of NEF2L2 regulation. Multi-omics clustering revealed four distinct clusters, variably enriched for place of origin, gender, and mutation status. Extensive characterization of the immune landscape of LUADs identified potential therapeutic vulnerabilities including CTLA4 and IDO1. An STK11-enriched cluster had a notably "cold" immune landscape; neutrophil degranulation was proposed as a mechanism for this immune regulation. Kinase outlier analyses suggested novel therapeutic possibilities, while tumor-normal analyses defined candidate diagnostic biomarkers, cancer testis antigens and other neoantigens, and helped illuminate carcinogenesis. These and other analyses are intended to provide new insights into LUAD biology and facilitate testable therapeutic hypotheses, including for the development of targeted chemo- or immuno-therapies. Furthermore, this diverse, densely

characterized and closely annotated sample population provides a vast dataset that should be an important resource for the lung cancer and broader scientific communities.

This abstract is also being presented as Poster A02.

PR12 N-803 plus nivolumab for advanced or metastatic non-small cell lung cancer: Update on phase II experience of combination PD1 blockade with an IL-15 superagonist. J. Wrangle[1], V. Velcheti[2], M. Patel[3], M. Sweiderska-syn[1], L. Macpherson[1], C. Coggins[1], C. Kreig[1], W. Redmond[4], A. Rock[5], J. Lee[5], M. Rubinstein[1]. [1]Medical University of South Carolina, Charleston, SC, [2]New York University, New York, NY, [3]University of Minnesota, Minneapolis, MN, [4]Earle A. Chiles Research Institute, Portland, OR, [5]ImmunityBio, Los Angeles, CA.

Immunotherapy has radically altered the treatment landscape of non-small cell lung cancer (NSCLC), yet the majority of patients treated with single-agent PD-1 immune checkpoint blockade (ICB) will not respond to these treatments. Among those who do respond, long-term survival is possible but modestly prevalent. Many NSCLC patients now receive chemo-immunotherapy as front-line treatment, exhausting the inventory of the most active agents against the disease in the first-line setting. New strategies to improve response rates and salvage therapeutic benefit at the time of progression on PD-1 ICB monotherapy or PD-1 ICB containing regimens are imperative. Common-gamma chain agonist cytokine immunotherapies have been in use in solid tumors as FDA-approved agents since 1992, yet their use remains restricted to specialty centers willing to offer inpatient administration of highly toxic doses of recombinant IL-2 in order to achieve rare clinical responses. IL-15, a member of the IL-2 common-gamma chain receptor family of cytokines, is a potent agonist for CD8+ T-cells and is the canonical growth factor for natural killer cells, yet it spares activation of the CD4+ compartment of T cells due to poor interaction with CD25. Here we present an updated experience of combining the IL-15-based superagonist N-803 with the PD-1 immune checkpoint blockade antibody nivolumab in patients with metastatic non-small cell lung cancer. Previously we have published the dose-finding experience and preliminary clinical results from the phase Ib portion (PMID 29628312) of this ongoing phase Ib/II trial. In addition to patients treated with the recommended phase II dose from the phase IB study, we also present the experience of alternate cytokine dosing schedules and the correlate work used to determine optimal administration. Uniquely important responders, including durable response after chemoimmunotherapy failure as well as potential biomarkers of response, will be discussed. This investigator-initiated clinical trial will conclude soon, but also discussed will be two follow-on industry-sponsored trials examining the combination in two NSCLC settings at a time of burgeoning interest in cytokine therapies.

This abstract is also being presented as Poster A37.

A02 Proteogenomic characterization reveals therapeutic vulnerabilities in lung adenocarcinoma.
M. A. Gillette[1], S. Satpathy[1], S. Cao[2], S. Dhanasekaran[3], S. Vasaikar[4], K. Krug[1], F. Petralia[5], Y. Li[2], W.-W. Liang[2], B. Reva[5], R. Hong[6], S. Savage[7], G. Getz[1], Q. K. Li[8], B. Zhang[7], H. Rodriguez[9], K. Ruggles[6], A. I. Robles[9], K. C. Clauser[1], R. Govindan[2], P. Wang[5], A. Nesvizhskii[3], L. Ding[2], D. R. Mani[1], S. A. Carr[1]. [1]Broad Institute of MIT and Harvard, Cambridge, MA, [2]Washington University in St. Louis, St. Louis, MO, [3]University of Michigan, Ann Arbor, MI, [4]University of Texas MD Anderson Cancer Center, Houston, TX, [5]Icahn School of Medicine at Mt. Sinai, New York, NY, [6]NYU School of Medicine, New York, NY, [7]Baylor College of Medicine, Houston, TX, [8]Johns Hopkins University School of Medicine, Baltimore, MD, [9]National Cancer Institute, Bethesda, MD.

This abstract is being presented as a short talk in the scientific program. A full abstract is printed in the Proffered Abstracts section (PR11) of the Conference Proceedings.

A03 Lung adenocarcinoma resident microbiome may contribute to cancer hypomethylation status.
E. A. Marshall[1], E. A. Vucic[2], F. S. L. Filho[3], J. M. Leung[3], S. Lam[1], W. L. Lam[1]. [1]BC Cancer Research Centre, Vancouver, BC, Canada, [2]New York University School of Medicine, New York, NY, [3]UBC Centre for Heart Lung Innovation, Vancouver, BC, Canada.

Lung cancer is a devastating disease, and is responsible for the greatest fraction of cancer-associated deaths worldwide. Human lungs were long thought to be sterile, but as a barrier organ, are colonized by numerous bacterial communities. Here, we sought to characterize the lung adenocarcinoma (LUAD) microbiome and determine if it plays a role in tumor behaviour. After patient consent, paired LUAD tumors and adjacent nonmalignant tissues (NM, n=77) were obtained. Extracted DNA was sequenced (16S rRNA V4 regions) using MiSeq. Methylation status of tumor tissue was determined by DNA bisulfite conversion and hybridization to the Illumina Human Methylation 27 array after tissue microdissection and DNA extraction. Methylation data were normalized, and average Beta values were compared by paired T-test. Validation of bacterial abundances was performed on publicly available whole RNA sequencing data depleted of reads aligning to the human genome (TCGA, 484 tumors and 58 NM). The potential functionality of the bacterial metagenome was assessed using the PICRUSt2 platform. When LUAD tumors are compared to NM tissue, we observe an increase in alphaproteobacteria, specifically *Bradyrhizobium* (p-adjusted=0.02). Conversely, a significantly lower abundance of gammaproteobacteria (*Acinetobacter*) is observed in the tumors, and an enrichment of this family is observed in NM samples (p-adjusted=0.03). Interestingly, we also observed a significant increase in *Deinococcus* in tumors (p=0.04; previously reported in LUSC). Using functional metagenome prediction, we observed a significant decrease in S-adenosyl-L-methionine synthesis (SAM; a global methyl donor) when tumors were compared to NM samples. In assessing the global patterns of DNA methylation in corresponding tissues, we observed hypomethylation of tumors compared to NM tissue genome-wide ($p<0.001$). To delineate the association of bacterial profiles with observed methylation patterns, we assessed tumor methylation data in the context of predicted SAM involvement. Indeed, tumors with high predicted SAM biogenesis in their microbiome had significantly more methylated regions than those with low involvement (high/low quartiles, p=0.002). Here, we assess the microbiome profile of LUAD and NM tissue and find that LUAD is enriched in alphaproteobacteria and deficient in gammaproteobacteria. In tumors, we find that downregulation of SAM biogenesis in the bacterial population, potentially as a result of intratumoral selection pressure, is associated with patterns of global hypomethylation in lung cancer.

A04 Lung-resident microbial signature precedes signs of lung malignancy.
E. A. Marshall[1], F. S.L. Filho[2], D. D. Sin[2], S. Lam[1], J. M. Leung[2], W. L. Lam[1]. [1]BC Cancer Research Centre, Vancouver, BC, Canada, [2]UBC Centre for Heart Lung Innovation, Vancouver, BC, Canada.

Shifts in the microbial populations that colonize human tissues have been shown to affect host biologic pathways. In fact, changes in the lung-epithelial-resident microbiota have been associated with various lung diseases. In cancers in general, specific bacteria have been shown to confer increased risk of disease (e.g., *H. pylori* in gastric cancer). In lung cancer, the tumor microbiome has been shown to be less diverse than normal tissue, but the effect of microbial composition alterations in airways prior to diagnosis of lung cancer is unknown. We sought to characterize the microbiome in airways of patients found to have lung cancer on follow-up. Following consent, bronchial brushes were obtained from 48 patients at a high risk of lung cancer. With a mean follow-up of 9.4+-1.2 years, 5/48 were diagnosed with lung cancer, and 3/48 were diagnosed with lung cancer at bronchoscopy. 16S sequencing was performed on bronchial epithelial taken from the airways of each patient, and the QIIME2 platform was used to classify

the bacterial populations. The bacterial taxonomy, alpha, and beta diversity measures were compared according to cancer status, and bacterial metagenome functionality was assessed using PICRUSt2. We found that patients with lung cancer and those who would develop it had lower airway bacterial diversity. Further, individuals who developed lung cancer over time displayed significantly different airway microbiome profiles from those who did not, but similar profiles to those who already had cancer ($p<0.0001$), with global taxonomic shifts observable at the phylum level. Using gene content inference, we observed that the lung-resident bacterial communities of patients with prevalent and incident cancers had significantly different metabolic profiles when compared to patients with no cancer. In particular, we observed an enrichment in the metabolites associated with cancer pathway (Wnt and Notch) activation (p-adjusted<0.0001), implicating a role of lung-resident bacterial communities in cancer initiation. Validation in an independent cohort consisting of 55 incident cancer, 18 prevalent cancer, and 263 noncancer subjects is ongoing. Here, we profile the microbial community resident to the lung epithelium and detect changes in this community years prior to the clinical detection of lung cancer. This work lays a foundation for further prospective studies leveraging microbiome profiles to further our understanding of the role of the lung microbiome in the pathogenesis of lung cancer.

A05 ART1, a mono-ADP-ribosyltransferase, regulates tumor-infiltrating CD8+ T cells and is highly expressed in EGFR mutated lung cancers. Sumit Mukherjee[1], Erik Wennerberg[1], Clarey Hung[1], Najla Saadallah[1], Shashi Kariyawasam[1], Mohamed Kamel Hussein[2], Navneet Narula[3], Prasad Adusumilli[4], Alain Borczuk[1], Nasser Altorki[1], Timothy McGraw[1], Brendon M. Stiles[1]. [1]Weill Cornell Medicine, New York, NY, [2]Central Michigan University College of Medicine, Mt. Pleasant, MI, [3]New York University, New York, NY, [4]Memorial Sloan Kettering Cancer Center, New York, NY.

Introduction: ADP-ribosyltransferase 1 (ART1), a GPI-linked cell surface protein, is broadly expressed at the protein level in human tumors and has been linked to tumor progression in colon cancer and gliomas. ART1 may regulate the immune microenvironment through mono-ADP-ribosylation of the P2X7 receptor on CD8+ T cells, leading to T-cell apoptosis through NAD-induced cell death. P2X7R expression is prominent on tissue resident memory (Trm) CD8+ T cells, which have increasingly been recognized for their critical role in immune response. We evaluated the role of ART1 in an immune-competent murine model and sought to determine the expression of ART1 in human tumors, particularly EGFR mutated tumors, which are known to be poorly responsive to immunotherapy.

Methods: Initial experiments were performed using the KP1 lung cancer cell line derived from lung tumors of KRASG12D/P53-/- mice. KP1-shART1 cells with doxycycline (DOX)-inducible knockdown of ART1 were generated by lentiviral constructs and used in flank and tail vein tumor models to determine ART1 effects on tumor growth. A lung adenocarcinoma tissue microarray (TMA) was then stained and scored for ART1 expression in order to determine ART1 expression in human tumors. We also evaluated ART1 expression in patient samples by whole-tumor RNAseq and IHC from a prospective clinical trial of neoadjuvant durvalumab +/- subablative radiation therapy (NCT02904954).

Results: ART1 knockdown significantly decreased tumor burden in flank and tail vein tumor models. Populations of tumor-infiltrating CD8+ T cells and of P2X7R+/CD8+ T cells were higher with ART1 knockdown, suggesting that ART1 expression on tumor cells may regulate tumor-infiltrating T cells. In human lung cell lines, the EGFR+ cell line H1650 expressed significantly more cell surface ART1 than A549 cells or BEAS cells (2.7- and 6.9-fold, respectively). In the TMA, among 463 stage I patients, 257 patient tumors (55.5%) strongly expressed ART1. Among patients with EGFR mutated tumors (n=79), 69.6% strongly expressed ART1 compared to 52.9% of KRAS+ tumors (n=119, p=0.03). In NCT02904954, among patients with whole-tumor RNAseq performed from preoperative biopsy (n=21), relative ART1 expression was 3-fold higher in EGFR+ patients (n=6). Median post-treatment H-scores for ART1 staining in resected tumors were also higher in EGFR+ tumors (120 vs. 77.5, $p<0.065$). No patients with EGFR+ tumors (n=8) had a major pathologic response to neoadjuvant durvalumab +/- RT, compared to a 38% combined arm MPR rate in EGFR- tumors (n=34). P2RX7 was strongly expressed in post-treatment tumors by whole-tumor RNAseq and trended higher in responders.

Conclusions: ART1 expression on lung cancer cells modulates tumor-infiltrating CD8 T cells. Knockdown of ART1 abrogates tumor growth, suggesting that ART1 may be a potential novel immune checkpoint and a therapeutic target. ART1 is particularly overexpressed in EGFR mutated lung cancers and may provide one mechanism to help explain their poor response to immunotherapy.

A06 Tri-complex inhibitors of the oncogenic, GTP-bound form of KRASG12C overcome RTK-mediated escape mechanisms and drive tumor regressions in preclinical models of NSCLC. R. Nichols, C. Schulze, A. Bermingham, T. Choy, J. Cregg, G. Kiss, A. Marquez, D. Reyes, M. Saldajeno-Concar, C. Weller, D. Whalen, Y. Yang, Z. Wang, E. S. Koltun, M. Singh, D. Wildes, A. L. Gill, R. Hansen, S. Kelsey, M. Goldsmith, J. Smith. Revolution Medicines, Redwood City, CA.

RAS proteins are small GTPases that drive cell proliferation and survival when bound to GTP. Mutant RAS proteins are found in approximately one third of NSCLC, and exist predominantly in the GTP-bound state, leading to aberrant downstream signaling via interaction with effectors such as RAF. Recently, multiple potent, covalent inhibitors of the oncogenic mutant KRASG12C have entered development and are driving high lung cancer response rates in early clinical trials. These inhibitors target the inactive, GDP-bound form of KRASG12C, KRASG12C(OFF), and thus rely on the residual intrinsic hydrolysis of GTP to cycle KRASG12C proteins through the GDP-bound state. This mechanism is vulnerable to adaptive responses in cancer cells that increase upstream signaling to further elevate the relative abundance of the active, GTP-bound state, KRASG12C(ON). An inhibitor that directly targets KRASG12C(ON) would overcome this limitation. We have developed tri-complex inhibitors of KRASG12C(ON) that promote a ternary complex between KRASG12C and the immunophilin cyclophilin A (CypA). KRASG12C(ON) inhibitors attenuate both RAS-MAPK signaling and cell viability in cancer cell lines bearing KRASG12C mutations. In vivo administration of a KRASG12C(ON) inhibitor drives dose-dependent tumor regressions in the NCI-H358 KRASG12C NSCLC xenograft mouse model and is well tolerated. Consistent with targeting the KRAS(ON) state, inhibitory activity in vitro is unaffected by RTK activation, whereas the activity of first-generation KRASG12C(OFF) inhibitors is significantly attenuated. In addition, proliferation of NCI-H358 cells in vitro is suppressed for a significantly longer duration with KRASG12C(ON) inhibitor treatment compared to KRASG12C(OFF) inhibitors. The ability to target the GTP-bound form of mutant KRAS permits a broad array of combination opportunities. Combination of KRASG12C(ON) inhibitors with agents targeting pathway nodes both up- and downstream of RAS, as well as other parallel pathways, can drive combination benefit in distinct cancer histotypes. Tri-complex inhibitors that target the active, GTP-bound form of KRAS thus represent a second generation of KRASG12C inhibitor. Chemical modulation of the noncovalent and covalent interactions of our tri-complex inhibitors provides an exciting opportunity to step beyond KRASG12C to target the GTP-bound state of additional RAS variants, and we demonstrate in vitro covalent inhibition of KRASG13C.

A07 The genomic landscape of SMARCA4 alterations and association with patient outcomes in lung cancer. A. J. Schoenfeld, J. Montecalvo, A. Namakydoust, J. Lavery, C. Bandlamudi, H. Rizvi, S. Paul, M. Arcila, J. Chang, J. Sauter, A. Beras, M. Ladanyi, B. Taylor, M. Donoghue, G. Heller, M. Hellmann, N. Rekhtman, Gregory Riely. Memorial Sloan Kettering Cancer Center, New York, NY.

Background: Genomic abnormalities in the subunits of the SWI/SNF (Switch/Sucrose NonFermentable) chromatin remodeling complex occur in approximately 20% of solid tumors. The tumor suppressor SMARCA4 is the most commonly altered gene within the SWI/SNF chromatin remodeling complex in lung cancer, but its relationship to other genomic abnormalities and clinical impact is unknown.

Methods: We evaluated all non-small cell lung cancer patients with SMARCA4 alterations detected by MSK-IMPACT next-generation sequencing (NGS) and who were treated at Memorial Sloan Kettering Cancer Center (MSK). A cohort of patients with metastatic non-small cell lung cancer who had MSK-IMPACT without SMARCA4 alterations and were treated during the same time period was used as a compare group. Clinical and molecular features were compared to examine how SMARCA4 alterations relate to molecular phenotype, and in patients treated with immune checkpoint inhibitors (ICIs), we assessed how these interactions impacted efficacy.

Results: We identified 404 of 4,813 NSCLC patients (8% of NSCLCs) with SMARCA4-mutant lung cancer. We found that the presence of SMARCA4 abnormalities is enriched in patients with KRAS, STK11, and KEAP1 mutations, but independently and additively shortens overall survival with these co-occurring alterations. Based on SMARCA4 protein expression and site of SMARCA4 mutations, we describe two distinct classes of SMARCA4 alterations associated. We also found that treatment with ICIs is associated with improved outcomes in patients with SMARCA4-mutant tumors and the class of mutations associated with protein loss correlates with increased response to ICIs.

Conclusion: SMARCA4 alterations are associated with KEAP1, STK11, and KRAS mutations in patient with

NSCLC, but individually represent a novel negative prognostic biomarker. Despite association with poor outcomes, SMARCA4-mutant lung cancers may also be uniquely sensitive to immunotherapy.

A08 MYC-driven SCLC has unique metabolic vulnerabilities. S. J. Wait[1], M. D. Chalishazar[1], F. Huang[2], J. S. Bomalaski[3], R. J. DeBerardinis[2], T. G. Oliver[1]. [1]Huntsman Cancer Institute, Salt Lake City, UT, [2]University of Texas Southwestern Medical Center, Dallas, TX, [3]Medical Affairs, Polaris Group, San Diego, CA.

Small-cell lung cancer (SCLC) is a highly aggressive neuroendocrine lung tumor that has been treated clinically as a homogeneous disease. Recent discoveries suggest that SCLC is heterogeneous with distinct molecular subtypes. Whether metabolic differences exist among SCLC subtypes is largely unexplored. We have aimed to determine whether metabolic vulnerabilities exist between SCLC subtypes that can be therapeutically exploited. Toward this end, we performed steady-state metabolomics on tumors isolated from distinct genetically engineered mouse models (GEMMs) representing the MYC and MYCL-driven subtypes of SCLC. We discovered that SCLC subtypes driven by different MYC family members have distinct metabolic profiles. Purine nucleotide biosynthesis and arginine/urea cycle pathways were enriched specifically in MYC-driven SCLC (Huang et al., Cell Metab 2108; Chalishazar et al., Clin Can Res 2019). MYC-driven SCLC preferentially depends on arginine-regulated pathways for polyamine biosynthesis and mTOR pathway activation. Chemoresistant SCLC cells exhibited increased MYC expression and similar metabolic liabilities as chemo-naive MYC-driven cells. Arginine depletion with pegylated arginine deiminase (ADI-PEG20) dramatically suppressed tumor growth and promoted survival of mice specifically with MYC-driven tumors, including in GEMMs, human cell line xenografts, and in new patient-derived xenograft (PDX) models. ADI-PEG20 was significantly more effective than the standard-of-care chemotherapy in GEMMs; however, tumors eventually relapse and acquire resistance to ADI-PEG20. Our current efforts are focused on identifying mechanisms of ADI-PEG20 resistance. We find that expression of the arginine biosynthetic enzyme ASS1 is frequently induced in ADI-PEG20 relapsed tumors in mouse and PDX models. Metabolite profiling of ADI-PEG20-resistant tumors suggests that ASS1 induction is associated with metabolic rewiring, which we predict will be associated with new metabolic vulnerabilities. Pathway analyses of metabolite data are consistent with the notion that ASS1 induction causes increased consumption of aspartate to generate arginine, and thereby ameliorate the demand for exogenous arginine. We predict that the diversion of aspartate away from nucleotide biosynthesis will lead to increased demand on other metabolic pathways for nucleotide biosynthesis. Preliminary data have identified pathways whose inhibition may cooperate with ADI-PEG20 to further extend the survival of mice with MYC-driven SCLC.

A09 Transcriptional subtypes resolve tumor heterogeneity and identify therapeutic vulnerabilities in lung cancer. A. Daemen[1], J. E Cooper[2], S. Myrta[3], M. Wongchenko[2], E. Lin[2], J. E Long[2], O. Foreman[2], Z. Modrusan[2], J. Tremayne[2], C. C de la Cruz[2], M. Merchant[2], S. E Martin[2], Y. Yan[2], M. R Junttila[1]. [1]ORIC Pharmaceuticals, South San Francisco, CA, [2]Genentech, Inc., South San Francisco, CA, [3]Roche, Warsaw, Poland.

This abstract is being presented as a short talk in the scientific program. A full abstract is printed in the Proffered Abstracts section (PR01) of the Conference Proceedings.

A10 A novel inhibitor for KRASG12C mutant lung carcinoma. H. Y. Khan[1], Y. Li[1], A. Aboukameel[1], G. Mpilla[1], R. Sexton[1], T. Kanbur[2], H. Cetinkaya[2], A. Cagir[2], M. N. Al-Hallak[1], A. Sukari[1], A. S. Azmi[1], M. Nagasaka[1]. [1]Karmanos Cancer Institute/Wayne State University, Detroit, MI, [2]Izmir Institute of Technology, Izmir, Turkey.

Background: Mutations in KRAS are among the most common aberrations in cancer. However, despite considerable research efforts, KRAS remains a challenging therapeutic target. In recent years, there has been a drive to develop KRAS mutant specific drugs. Among the different known mutations, the KRASG12C (glycine 12 to cysteine) has been considered druggable. Studies have shown that due in part to the close proximity of Cysteine 12 to both the nucleotide pocket and the switch regions, thiol-reactive compounds can bind to the active site covalently and inhibit KRASG12C mutation-driven signaling. The absence of this particular cysteine residue in wild-type KRAS makes such an approach very selective towards cancer cells. We have discovered that derivatives of 6-(naphthale-1-yl)-5,6-dihydro-2H-pyran-2-one (klavuzon) have potent inhibitory effects over KRASG12C due to their thiol-reactive property.

Methods: We compared the antitumor activity of klavuzon derivatives (TK-126, TK421, HC-70-1, HC-

01-155, and HC-01-183) to commercially available KRASG12C inhibitors of MRTX 1257, ARS 1620, and AMG 510 against a panel of KRASG12C, KRASG12D, KRASG12V, and KRAS wild-type cell lines of lung cancer and NCI isogenic RAS-Less MEFs with different KRAS mutations. The antitumor activity was assessed in KRASG12C vs. KRASG12D cell line pair derived subcutaneous and ERK1/2 overexpressing patient derived xenograft.

Results: Klavuzon derivatives showed KRASG12C selective activity sparing other mutants or KRAS wild-type cells (IC50 several-fold higher). The antitumor activity was comparable to commercially available KRASG12C inhibitors. The drugs suppressed colony formation and disintegrated spheroids with concurrent induction of apoptosis and cell cycle arrest in KRASG12C cell lines. Molecularly, klavuzon treatment resulted in suppressed ERK and p-ERK expression specifically in KRASG12C cells, indicating target engagement. Klavuzon derivates showed synergy with shp2 inhibitor. In xenograft studies, potent antitumor activity in pERK overexpressing patient-derived tumors was observed. The antitumor activity of lead inhibitor is currently being evaluated in KRASG12C vs. KRASG12D cell line-derived xenograft.

Conclusions: Klavuzon derivatives demonstrate selectivity against KRASG12C mutant cell lines in vitro and show antitumor activity against p-ERK1/2 and overexpressing patient-derived xenograft sparing wt-KRAS and KRASG12D cell lines. Our preclinical studies are anticipated to bring forward a new and personalized therapy for the so far incurable mutant KRAS-driven tumors.

A11 Blockade of myeloid suppressor cells overcomes the anti-PD-1/PD-L1 resistance in KRAS-driven and LKB1-deficient NSCLC. R. Li[1], R Salehi-Rad[1], M. Momcilovic[1], R. Lim[1], S. Ong[1], Z. Huang[1], L. Tran[1], J. Zhe[1], M. Paul[1], M. Teitell[1], J. Minna[2], K. Krysan[1], D. Shackelford[1], B. Liu[1], S. Dubinett[1]. [1]University of California Los Angeles, Los Angeles, CA, [2]UTSW, Dallas, TX.

This abstract is being presented as a short talk in the scientific program. A full abstract is printed in the Proffered Abstracts section (PR06) of the Conference Proceedings.

A12 The SHP2 inhibitor RMC-4630 in patients with KRAS-mutant non-small cell lung cancer: Preliminary evaluation of a first-in-man phase 1 clinical trial. S. I. Ou[1], M. Koczywas[2], S. Ulahannan[3], P. Janne[4], J. Pacheco[5], H. Burris[6], C. McCoach[7], J. S. Wang[8], M. Gordon[9], E. Haura[10], J. W. Riess[11], V. Zhu[1], K. Ng[4], S. G. Eckhardt[12], A. Capasso[12], R. Dua[13], A. Chen[13], Z. Wang[13], J. Hayes[13], R. Nichols[13], T. Bivona[7]. [1]University of California Irvine, Irvine, CA, [2]City of Hope Hospital, Duarte, CA, [3]University of Oklahoma, Norman, OK, [4]Dana-Farber Cancer Institute, Boston, MA, [5]University of Colorado Denver, Denver, CO, [6]Sarah Cannon Research Institute, Nashville, TN, [7]University of California San Francisco, San Francisco, CA, [8]Florida Cancer Specialists, Fort Myers, FL, [9]Honor Health, Scottsdale, AZ, [10]Moffitt Cancer Center, Tampa, FL, [11]University of California Davis, Davis, CA, [12]University of Texas Austin, Austin, TX, [13]Revolution Medicines, Redwood City, CA.

This abstract is being presented as a short talk in the scientific program. A full abstract is printed in the Proffered Abstracts section (PR02) of the Conference Proceedings.

A13 A functional genomics approach highlights new therapeutic opportunities for KRAS-mutated non-small cell lung cancer. F. Reggiani[1], E. Sauta[2], G. Gobbi[1], B. Donati[1], I. Faria Do Valle[3], F. Torricelli[1], D. C. Ambrosetti[4], A. Ciarrocchi[1], V. Sancisi[1]. [1]AUSL-IRCCS di Reggio Emilia, Reggio Emilia, Italy, [2]University of Pavia, Pavia, Italy, [3]Northeastern University , Boston, MA, [4]University of Bologna, Bologna, Italy.

Despite the introduction of innovative therapeutics, the prognosis of non-small cell lung cancer (NSCLC) remains poor, with an overall survival at five years of only 16%. In recent years, a great effort has been conferred to target oncogenes on which cancer cells rely for survival and proliferation. However, the success of this strategy is often limited by development of drug resistance and by difficult-to-target oncogenes. KRAS-driven lung adenocarcinoma is particularly hard to target, still representing an unmet clinical need and an open challenge. In this context, based on the notion that tumors rely for their survival also on genes that are not classical oncogenes, an innovative strategy is to move the focus from oncogenes to “non-oncogene addiction.” Because of their aberrant biology, cancer cells are more sensitive than normal cells to inhibition of those nononcogenic pathways. In this work, we aimed to identify nononcogene dependencies that can be exploited to develop novel therapeutic strategies for KRAS-mutated NSCLC. To this end, we used a CRISPR/

Cas9 genome-scale knockout approach in KRAS-mutated NSCLC cells. After normalization with CERES algorithm, 705 genes were identified as nononcogene addictions. Next, we compared our results with data available through the Cancer Dependency Map Portal (DepMap), which collects dependency data of 73 lung cancer cell lines. From this analysis, we obtained two outputs: a list of common dependencies in lung cancer cell lines and a list of KRAS-mutated NSCLC-specific vulnerabilities. Reactome enrichment analysis on these genes identified pathways related to mRNA metabolism as key dependencies. We showed that a subset of these genes is overexpressed in tumor samples and associated with worse prognosis in adenocarcinoma patients. These candidates represent excellent therapeutic targets. Starting from our lists of essential genes, we also identified already available chemical compounds that inhibit the activity of those genes. Some of the drugs are already approved or currently in clinical trials for NSCLC, supporting the validity of our analysis. Intriguingly, we also identified druggable genes whose role in lung carcinogenesis is controversial or has been poorly investigated. These drug-target interactions may be used to reposition already available drugs for NSCLC treatment. Through a functional genomics strategy, we highlighted novel KRAS-mutated NSCLC vulnerabilities that can be used both for drug repurposing and for developing new therapeutics.

A14 Circulating ensembles of tumor-associated cells are ubiquitous in lung cancers. Dadasaheb B. Akolkar[1], Sewanti Limaye[2], Darshana Patil[1], Pradip Fulmali[1], Pooja Fulmali[1], Sachin Apurwa[1], Sushant Pawar[1], Vineet Datta[1], Cynthe Sims[1], Ajay Srinivasan[1], Rajan Datar[1]. [1]Datar Cancer Genetics Limited, Nasik, Maharashtra, India, [2]Kokilaben Dhirubhai Ambani Hospital, Mumbai, India.

Detection of lung cancers is based on histopathologic analysis of tumor tissue obtained by invasive biopsies following findings on low-dose computed tomography (LDCT) or other symptomatic presentation in suspected cases. There is presently no noninvasive nonradiologic blood-based test with high specificity and sensitivity for detection of lung cancers. Considering that unprovoked thromboembolism is a significant risk in multiple cancers, we hypothesized that tumor-derived circulating emboli in peripheral blood could comprise cancer cells and would serve as a reliable biomarker for detection of lung cancers. These circulating ensembles of tumor-associated cells (C-ETACs) are defined as clusters of 3 or more cells of tumorigenic origin (EpCAM+, CK+, and CD45±). We obtained 15ml of blood from 11,063 individuals, including 438 cases of non-small cell lung cancer (NSCLC) as well as from 10,625 asymptomatic individuals with age-related elevated risk, prior to LDCT scan. PBMC were isolated by centrifugation. C-ETACs were enriched using an epigenetically activated medium that eliminates normal cells (nontumorigenic origin) and confers survival privilege on apoptosis-resistant cells of tumorigenic origin (C-TACs, circulating tumor-associated cells) and their clusters (C-ETACs). Surviving C-ETACs were confirmed by immunostaining (EpCAM, pan-CK, CD45, TTF-1, Napsin-A). C-ETACs were detected in 374 (85.4%) of 438 lung cancers irrespective of extent (stage/metastatic status) of disease and prior treatments. Among the 587 asymptomatic individuals with suspicious findings on LDCT (Lung RADS category ≥ 2), C-ETACs were detected in 21 individuals (3.6%). Among the 10,038 asymptomatic individuals with no suspicious findings on LDCT (Lung RADS = 1), C-ETACs were detected in 371 individuals (3.7%). C-ETACs were ubiquitous in NSCLC regardless of extent and treatment status and pose significant latent risk of metastasis/recurrence. Simultaneously, the relative undetectability of C-ETACs in asymptomatic cohort indicates causative connection of C-ETACs with lung malignancies. C-ETACs are suitable for screening suspected populations for lung cancers.

A16 Autoantibody-antigen complexes can detect limited-stage small-cell lung cancer. K. J. Lastwika[1], Y. Zhang[1], J. J. Ladd[1], P. P. Massion[2], A. M. Houghton[1], P. D. Lampe[1]. [1]Fred Hutchinson Cancer Research Center, Seattle, WA, [2]Vanderbilt University, Nashville, TN.

Small-cell lung cancer (SCLC) claims 30,000 American lives each year with five-year survival rates of just ~7%. Somewhat lost in these dismal statistics is the fact that patients diagnosed early (limited stage) display vastly superior survival metrics when compared to those diagnosed late (extensive stage). Since nearly 20% of limited-stage SCLC can be cured with conventional cytotoxic chemotherapy and/or surgery, earlier diagnosis could have a clinical impact. Unfortunately, the computed tomography screening approaches capable of early detection for non-small cell lung cancer (NSCLC) have not proved effective for SCLC. We have found that overall levels of plasma autoantibody-antigen complexes are >2x higher in SCLC compared to other cancer types including NSCLC, colon, breast, and pancreas cancer. Thus, we hypothesized that a blood-based autoantibody test could reliably detect SCLC while still at limited stage. Using high-density antibody arrays, we discovered and twice validated 8 IgG and 11 IgM highly specific autoantibody-antigen complexes for SCLC in 3

independent cohorts (1 prediagnostic and 2 diagnostic, total N=240). Using optimized logistic regression, we identified 4 autoantibody-antigen complexes that performed well in each study with an AUC of 0.915 (53% sensitivity at 90% specificity) in the prediagnostic set, 1.0 (100% sensitivity at 90% specificity) in the first diagnostic cohort and 0.866 (64% sensitivity at 90% specificity) in the second diagnostic cohort. Panel autoantibodies were similarly effective when the plasma was drawn up to 1 year prior to diagnosis, at limited-stage diagnosis, or at extensive-stage diagnosis. We have evidence that each panel autoantibody is specific for SCLC as none are upregulated in NSCLC (N=45) samples or in other comorbidities examined, including COPD (N=31) and autoimmunity (N=15). Our findings suggest these autoantibodies have the potential to be used at the time of lung cancer screening to identify limited-stage SCLC to increase the survival of this recalcitrant cancer.

A17 Inhibition of RUVBL1/2 ATPase activity drives immune infiltration and radiosensitizes non-small cell lung cancer. Paul Yenerall[1,2], Amit K. Das[2], Shan Wang[1,3], Rahul K. Kollipara[1], Huiyu Li[2], Long Shan Li[2], Pamela Villalobos[4], Josiah Flaming[2], Kenneth Huffman[2], Brenda C. Timmons[2], Collin Gilbreath[3], Rajni Sonavane[3], Jaime Rodriguez-Canales[5], Cesar Moran[5], Carmen Behrens[6], Makoto Hirasawa[7], Takehiko Takata[8], Ryo Murakami[9], Koichi Iwanaga[8], Ganesh V. Raj[2,3,10,11], Ignacio I. Wistuba[4,6], John D. Minna[2,10,11,12], Ralf Kittler[1,10,11]. [1]Eugene McDermott Center for Human Growth and Development, UT Southwestern Medical Center, Dallas, TX, [2]Hamon Center for Therapeutic Oncology Research, UT Southwestern Medical Center, Dallas, TX, [3]Department of Urology, UT Southwestern Medical Center, Dallas, TX, [4]Department of Translational Molecular Pathology, University of Texas MD Anderson Cancer Center, Houston, TX, [5]Department of Pathology, University of Texas MD Anderson Cancer Center, Houston, TX, [6]Department of Thoracic/Head and Neck Medical Oncology, University of Texas MD Anderson Cancer Center, Houston, TX, [7]Drug Metabolism & Pharmacokinetics Research Laboratories, Daiichi-Sankyo Co., Ltd., Tokyo, Japan, [8]Oncology Medical Science Department, Medical Affairs, Daiichi-Sankyo Co., Ltd., Tokyo, Japan, [9]Oncology Research Laboratories II, Daiichi-Sankyo Co., Ltd., Tokyo, Japan, [10]Department of Pharmacology, UT Southwestern Medical Center, Dallas, TX, [11]Harold C. Simmons Comprehensive Cancer Center, UT Southwestern Medical Center, Dallas, TX, [12]Department of Internal Medicine, UT Southwestern Medical Center, Dallas, TX.

Purpose of Study: Prior work in non-small cell lung cancer has shown that RUVBL1 and RUVBL2 (herein RUVBL1/2) are overexpressed in patient tumors and high expression predicts poor patient prognosis. Additionally, the inhibition of RUVBL1/2 ATPase activity using small molecules has shown modest activity as a monotherapy in some preclinical models. In this study we evaluated what clinically relevant agents could be combined with RUVBL1/2 inhibitors to improve therapeutic efficacy in NSCLC.

Experimental Procedures: Patient-derived NSCLC lines were treated with radiation, chemotherapy, and targeted inhibitors in combination with a RUVBL1/2 inhibitor, and viability was measured to determine synergy/potentiation using Combenefit (Bliss and Loewes synergy). Both the kinetics and magnitude of DNA damage after RUVBL1/2 inhibition and radiation was measured using immunofluorescence and Western blot in NSCLC and normal human bronchial epithelial cells. Humanized mice bearing NSCLC xenografts were treated with a RUVBL1/2 inhibitor and intratumoral immune infiltrate was measured using flow cytometry. Activation of the cGAS/STING pathway was monitored after RUVBL1/2 inhibitor treatment in NSCLC lines using Western blot.

Summary: Patient-derived NSCLC lines were treated with clinically relevant chemotherapies, targeted inhibitors, or radiation in combination with a highly specific RUVBL1/2 inhibitor or its enantiomer control and cell viability was measured. RUVBL1/2 inhibition significantly enhanced the killing of NSCLC following radiation, but not chemotherapy or other targeted agents. This enhancement was specific to NSCLC, not normal bronchial epithelial cells, and occurred by inhibiting the ability of NSCLC to efficiently repair their DNA. To gauge the effect of RUVBL1/2 inhibitors on the immune system, and thus potentially immunotherapy, humanized mice bearing NSCLC xenografts were treated with a RUVBL1/2 inhibitor. Treatment with a RUVBL1/2 inhibitor caused infiltration of T cells, B cells, and dendritic cells, suggesting that RUVBL1/2 inhibition stimulated the immune system. Additionally, treatment of NSCLC lines in vitro with a RUVBL1/2 inhibitor and radiation activates the cGAS/STING pathway, suggesting that RUVBL1/2 inhibitors could be combined with radiation and immunotherapy.

Conclusions: Inhibition of RUVBL1/2 as a monotherapy has modest efficacy due to a narrow therapeutic window. This work demonstrates that RUVBL1/2 inhibitors can enhance the cancer killing effect of radiation, but not other clinically relevant agents, specifically in tumor cells (i.e., spares normal cells).

Additionally, we show that treatment with a RUVBL1/2 inhibitor can cause immune infiltration in NSCLC tumors, and a RUVBL1/2 inhibitor in combination with radiation can activate the cGAS/STING pathway. In totality, our results suggest that further research should be done looking at the efficacy of RUVBL1/2 inhibitors with immune checkpoint inhibitors, both with and without radiation, in NSCLC.

A18 Culture of immortalized human alveolar epithelial cells in 2D and 3D to model lung adenocarcinoma progression in vitro. E. Tran, T. Shi, X. Liu, H. Wang, C. Marconett, B. Zhou, Z. Borok, I. A. Offringa. University of Southern California, Los Angeles, CA.

Background: Lung cancer is the leading cause of cancer death in the United States. Lung adenocarcinoma (LUAD) is the most common histologic subtype, arising from epithelial cells of terminal respiratory units called alveoli. The overall 5-year survival of lung cancer remains low at 19%. There is an urgent need to understand early events in LUAD development, as well as to develop new 1st- , 2nd- , and 3rd-line targeted therapies. Human organoids are powerful research tools for the molecular and mechanical manipulation of genetically diverse cells without exposing human subjects to treatment. Establishment of normal cell lines from human alveolar epithelial cells (AECs) has remained challenging due to the difficulty of growing primary cells in long-term culture. Human alveolar organoids would provide a powerful tool to 1) study LUAD development, progression, and drug resistance; 2) screen for new therapeutics; and 3) study the effects of environmental exposures on AECs.

Goal: Optimize growth and genetic conditions to derive human AEC lines from purified primary cells for the stepwise modeling of LUAD.

Approach: We tested different immortalization strategies using primary purified AECs to determine which condition allowed cells to continue proliferating while retaining their epithelial phenotype in two-dimensional (2D) culture and their ability to form spheroids in three-dimensional (3D) culture.

Results/Discussion: Using purified primary AECs from three deceased, deidentified human subjects, we found that the initial propagation of AECs in media containing Y-27632 and subsequent transduction with Simian virus 40 Large T antigen allowed cells to divide readily in 2D as a monolayer, while expressing epithelial marker E-cadherin but not mature lung genes. When placed in 3D Matrigel culture with fibroblasts, these cells form multilobulated structures expressing mature AEC markers, reminiscent of the peripheral lung. We are currently optimizing this system to allow stepwise modeling of LUAD.

A19 Epithelial beta 1 integrin regulates lung cancer susceptibility through NF-kB signaling. E. Plosa, J. Sucre, P. Gulleman, T. Blackwell. Vanderbilt University Medical Center, Nashville, TN.

Rationale: Cell-extracellular matrix (ECM) interactions are essential for maintenance of alveolar homeostasis in the adult lung. Alveolar epithelial cells (AECs) connect to the ECM through integrins. β1 integrin is the most common lung epithelial integrin subunit, and forms the receptors for collagen, laminin, and fibronectin. We have previously shown that epithelial β1 integrin regulates AEC inflammatory signaling during alveolar homeostasis. However, the role of epithelial β1 integrin during repair after injury remains undefined. We hypothesize that epithelial β1 integrin is required for alveolar repair by regulating AEC proliferation and survival in response to injurious stimuli.

Methods: We deleted β1 integrin in type 2 AECs in the adult lung in SP-C rtTA; TetO-Cre; β1f/f mice using doxycycline to induce Cre expression (called β1rtTA mice). Three-month-old β1rtTA and β1f/f mice were challenged with 3 ug/g intratracheal lipopolysaccharide (LPS) or PBS. β1f/f littermate mice and doxycycline naïve SP-C rtTA; TetO-Cre; β1f/f mice were used as controls and mice were crossed onto the CCR2-/- background. Lung slices obtained from β1rtTA and β1f/f lungs were cultured with the NF-kB inhibitor BAY-11-7082, LPS, or both.

Results: We previously reported that β1-deficient type 2 AECs are inflamed in the absence of injury, exhibited increased reactive oxygen species production, increased NF-kB activation, and secreting inflammatory cytokines. Aged 2-year-old β1rtTA mice exhibited chronic progressive macrophage dominant inflammation and developed adenocarcinoma. We next challenged 3-month-old β1rtTA mice with intratracheal LPS. LPS-treated β1rtTA mice had increased mortality at 7 days (43% β1rtTA vs. 91% β1f/f survival, $p < .05$) and an escalation in the number of recruited inflammatory cells from 24 hours to 7 days post-LPS challenge. In the β1rtTA survivors, histologic examination 21 days after LPS challenge resulted in emphysema in β1rtTA lungs, fibrotic regions identified by trichrome staining, and type 2 AEC hyperplasia. Histologic examination

6 months following LPS administration revealed sustained influx on monocyte-macrophages and early adenocarcinoma formation at 9 months of age in β1rtTA mice. Since the NF-kB pathway serves as a prosurvival mechanism, we treated ex vivo lung slices with an NF-kB inhibitor and LPS. In β1rtTA lung slices, NF-kB inhibition alone potentiated tissue remodeling and exacerbated AEC proliferation, suggesting that upregulated NF-kB plays a compensatory prosurvival role in the presence of chronically inflamed type 2 AECs. β1rtTA mice crossed to the CCR2 null background, which lack monocyte-macrophage recruitment, were protected from adenocarcinoma formation with age and following LPS challenge.

Conclusions: These findings suggest that β1 integrin relies on the prosurvival properties of the NF-kB pathway to regulate AEC proliferation during homeostasis and dysregulation of β1-mediated inflammation post injury increases cancer susceptibility.

A20 Estrogen metabolism in patients with EGFR-mutated and ALK-mutated non-small cell lung cancer (NSCLC). J. N. Bodor, J. Treat, D. D. Krzizike, C. L. Zawislak, L. Vanderveer, M. Yulis, A. Chau, E. A. Ross, A. J. Andrews, M. L. Clapper. Fox Chase Cancer Center, Philadelphia, PA.

Prior studies have shown that the human lung can extensively metabolize estrogen to reactive catechols. Of greatest concern is 4-hydroxyestrogen (4-OHE), a putative carcinogen, produced by cytochrome P450 1B1 (CYP1B1). In contrast, CYP1A1 metabolizes parent estrogens to 2-hydroxyestrogen (2-OHE), which are less reactive and converted to derivatives that may be antiproliferative. Data from this group strongly suggest that 4-OHE contributes to lung tumorigenesis, though its role in driver-mutated NSCLC has not been investigated. This study assessed estrogen metabolite profiles in EGFR-mutated and ALK-mutated NSCLC patients as compared to cancer-free subjects. Advanced-stage NSCLC patients with tumors that possessed either an EGFR (n = 14) or ALK (n = 8) mutation and cancer-free subjects (n = 17) were recruited from Fox Chase Cancer Center. Tumor mutation status of NSCLC patients was determined by tissue biopsy. All study participants were 50 years of age or older to circumvent any confounding influence of young age or premenopausal status on estrogen levels. NSCLC patients included 14 females and 8 males. Cancer-free subjects serving as controls were never-smoking women. Urine specimens were collected from study participants and urinary estrogen species (E1, E2, E3, 4-OHEs, 2-OHEs, 2-OMEs) were quantified using UPLC-MS/MS. Medians were calculated and the Wilcoxon rank-sum test was used to compare estrogen metabolite measures (4-OHEs/total estrogen, 2-OHE/total estrogen, and the ratio of 4-OHEs/2-OHEs) between NSCLC patients and control subjects. EGFR-mutated NSCLC patients had a significantly higher proportion of 4-OHEs/total estrogen (0.18 vs. 0.05, p-value = 0.048) and a trend towards lower 2-OHEs/total estrogen (0.18 vs. 0.26, p-value = 0.084) as compared to cancer-free control subjects. The ratio of 4-OHEs/2-OHEs was higher in EGFR-mutated NSCLC patients as compared to cancer-free controls (0.90 vs. 0.16, p = 0.053). Differences were not seen between ALK-mutated NSCLC patients and cancer-free subjects for the measures of 4-OHE/total estrogen (0.09 vs. 0.05, p-value = 0.842), 2-OHE/total estrogen (0.20 vs. 0.26, p-value = 0.238), and the ratio of 4-OHEs/2-OHEs (0.34 vs. 0.16, p-value = 0.669). The greater relative level of 4-OHE to 2-OHE in EGFR-mutated NSCLC patients suggests that enhanced production of 4-OHE may contribute to the development of EGFR-mutated lung tumors. Targeting CYP1B1, the enzyme responsible for 4-OHE production, may be of therapeutic interest. Research is ongoing to validate these findings in a larger cohort of EGFR-mutated NSCLC patients.

A21 Targeting glucose reliance in lung squamous cell carcinoma. M. Hsieh[1], S. Mazambani[2], J. Kim[2]. [1]UTSW, Dallas, TX, [2]UT Dallas, Richardson, TX.

This abstract is being presented as a short talk in the scientific program. A full abstract is printed in the Proffered Abstracts section (PR10) of the Conference Proceedings.

A22 Integrated proteometabolomic analysis reveals metabolic vulnerabilities in small-cell lung cancer. A. Prabhu[1], K. Scott[1], P. Stewart[1], D. Grass[1], M. Fernandez[1], J. Koomen[1], T. Bannister[2], S. Sumner[3], C. Rudin[4], G. Denicola[1], J. Cleveland[1], E. Haura[1]. [1]H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL, [2]The Scripps Research Institute, Jupiter, FL, [3]University of North Carolina, Chapel Hill, NC, [4]Memorial Sloan Kettering Cancer Center, New York, NY.

This abstract is being presented as a short talk in the scientific program. A full abstract is printed in the Proffered Abstracts section (PR04) of the Conference Proceedings.

A23 A genomically adjusted clinicopathologic model predicts recurrence in resected early-stage lung squamous cell carcinoma. J. G. Connolly, K. S. Tan, F. Sanchez-Vega, G. D. Jones, Y. Liu, R. Caso, G. Rocco, B. J. Park, P. S. Adusumilli, D. Molena, D. R. Jones. Memorial Sloan Kettering Cancer Center, New York, NY.

Introduction: In contrast to lung adenocarcinoma, identification of clinically relevant genomic perturbations in lung squamous cell carcinoma (LUSC) remains poorly characterized. Prognostic and therapeutic decisions following surgery in early and locoregionally advanced LUSC are almost exclusively driven by the TNM classification system, omitting high-risk clinicopathologic and tumor genomic information. To address this knowledge gap, we sought to determine if a combined clinicopathologic and genomic model could predict disease-free survival (DFS) better than traditional TNM assessments in completely resected LUSC.

Methods: A retrospective cohort study of a prospectively maintained database was performed for patients (N=95) with pathologic stage I-III LUSC who underwent complete resection from 2008-2018. Patients who received any induction therapy (N=9) were excluded. All patients had complete clinicopathologic data with broad-panel next-generation sequencing of the primary tumor, including matched controls to bioinformatically filter germline variants. DFS, the primary endpoint, and overall survival (OS) were estimated using Kaplan-Meier. Genomic pathway alterations (N=10) were determined as we previously reported. The prognostic model established associations between high-risk clinicopathologic variables and genomic alterations with DFS through Cox regression models. Concordance probability estimate (CPE) was used to discriminate performance between the existing TNM model and the developed prediction models.

Results: The median age was 70 years (range, 55-84), one-third were female (n=35), 55% (N=52) were pathologic stage I, and 98% (N=93) were ever-smokers. Median follow-up was 2 years. Recurrence occurred in 20% (19/95) and DFS was 74% (95% CI, 84-96%) at two years. Clinicopathologic features associated with DFS were lymphovascular invasion, visceral-pleural invasion, and pathologic stage. Tumor genomic analysis revealed alterations in the transcription factor BCL6 were independently associated with worse DFS (HR 5.23, 95% CI 1.73, 15.9, p=0.009), while mutations in the tumor suppressor ARID1A were associated with a worse OS (HR 2.98, 95% CI 0.91, 9.77, p=0.07). Pathway-centric analyses revealed no associations with our primary or secondary endpoints. Our prognostic clinicopathologic model outperformed the internally validated TNM model (CPE, 0.74 vs. 0.70) for prediction of DFS and our clinicopathologic model, adjusted for BCL6 genomic alterations, further improved discrimination (CPE=0.77).

Conclusions: We show that integration of high-risk clinicopathologic and tumor genomic profiling better predicts DFS than TNM classification alone in early-stage, surgically resected LUSC – an observation that may facilitate enrichment in future adjuvant therapy clinical trials. This exploratory genomic analysis also suggests future studies investigate putative therapeutic vulnerabilities in LUSC tumors harboring BCL6 and ARD1A genomic alterations.

A24 The genome-wide mutational landscape of lung cancer in never-smokers: The Women's Health Initiative (WHI) cohort. S. Moorthi, A. Paguirigan, G. Anderson, P. Porter, M. Holden, G. Ha, A. H. Berger. Fred Hutchinson Cancer Research Center, Seattle, WA.

This abstract is being presented as a short talk in the scientific program. A full abstract is printed in the Proffered Abstracts section (PR03) of the Conference Proceedings.

A25 PTPRH mutations in NSCLC regulate EGFR phosphorylation. M. R. Swiatnicki, J. P. Rennhack, E. R. Andrechek. Michigan State University, East Lansing, MI.

The dysregulation of receptor tyrosine kinases (RTK) has garnered plenty of interest within the cancer field, and attention has begun to turn to phosphatases regulating RTK behavior. Under normal cellular conditions, protein tyrosine phosphatases remove phosphate groups from tyrosine residues, thus maintaining signaling homeostasis. In whole-genome sequencing of primary mouse mammary tumors from the polyoma virus middle T antigen (PyMT) mouse model, we found a mutation in the protein tyrosine phosphatase receptor type H (Ptprh) gene. Targeted resequencing of 45 mouse tumors showed a conserved heterozygous or homozygous mutation present in 80% of tumors. This C>T mutation results in a valine-to-methionine shift within one of the fibronectin domains of PTPRH. Previous literature has shown interactions between PTPRH and epidermal growth factor receptor (EGFR). To determine the relevancy of PTPRH mutations in human cancer, data from The Cancer Genome Atlas (TCGA) were analyzed and revealed PTPRH mutations in 5% of non-small cell lung cancer (NSCLC) patients. Moreover, patients with a mutation in PTPRH were mutually exclusive from those with mutation or amplification of

EGFR. We hypothesize a mutation in PTPRH results in a failure of PTPRH to dephosphorylate EGFR, resulting in inappropriate maintenance of downstream signaling pathways important for proliferation and evading apoptosis. Since NCSLC patients with EGFR mutations are successfully treated with tyrosine kinase inhibitors (TKI), we also hypothesize tumors with a mutation in PTPRH will be sensitive to TKIs. In support of this, we demonstrated mouse tumors with a mutation in Ptprh had increased phosphorylated EGFR (pEGFR). Furthermore, CRISPR-mediated knockout of PTPRH in H23 NSCLC cells leads to increased pEGFR. Pathway signature analysis applied to microarray gene expression data from the Breast TCGA dataset (due to low sample size in the NSCLC dataset), and single sample gene set enrichment analysis applied to RNA sequencing data from the NSCLC TCGA dataset both predicted an increase in PI3K and AKT activity. This suggested the EGFR residue targeted by PTPRH was tyrosine 1197. Western blots on Ptprh mutant mouse tumors confirmed increased levels of pAKT. Additionally, immunohistochemistry for pEGFR 1197 revealed increased staining in mouse tumors with a mutation in Ptprh, with subcellular location in the nucleus rather than the membrane. To determine whether TKIs may be an effective treatment for NSCLC patients who harbor a PTPRH mutation, H1155 and H2228 NSCLC cell lines with PTPRH mutations in the fibronectin and phosphatase domains, respectively, were subjected to a dose response curve with the TKI osimertinib. These lines show significant growth differences as compared to the negative control cell line A427. While more work is needed to elucidate the role of mutant PTPRH in NSCLC, preliminary data suggest mutant PTPRH fails to dephosphorylate EGFR, and patients with a mutation in PTPRH may benefit from TKI therapy.

A26 Deciphering the functional redundancy of USP4 and USP15. S. Zachariah, J. Coulombe, M. Zhang, D. Gray. Cancer Therapeutics Program, Ottawa Hospital Research Institute, Ottawa, ON, Canada.

Ubiquitin-specific proteases (USPs) are a class of deubiquitinating enzymes that catalyze the removal of ubiquitin from various proteins and are involved in many cancers. Previous work established the USP paralogs USP4 and USP15 emerged from an ancestral USP about 500 million years ago from a whole genome duplication, and the majority of known vertebrate genomes retain a functional copy of both. USP4 was found to be consistently overexpressed in primary tumor tissue from small-cell carcinomas and adenocarcinomas of the lung. Despite their similarity, high expression of USP4 is correlated with decreased overall survival in lung adenocarcinoma, whereas high expression of USP15 is correlated with increased survival. Both USPs are known to be involved in some of the same signaling pathways such as Wnt/β-catenin; however, subfunctionalization has occurred such that they each regulate the stability of distinct substrates. To better understand each USP's role, we are analyzing mice in which one or both genes have been inactivated and have found that the absence of both USPs results in a lethal phenotype. Although USP4 and USP15 have diverged over evolutionary time, we hypothesize that there may still be some level of functional redundancy. We found that embryos null for both genes die at midgestation and are physically smaller than embryos heterozygous for both genes. They have underdeveloped livers, indicating a possible defect in hematopoiesis. Proper fetal hematopoiesis requires signaling through Wnt/β-catenin pathway, and a systematic analysis of the components of this pathway has been undertaken by Western blot and qPCR. Current data indicate that there are deficiencies in at least some USP4 substrates, and that the TCF transcriptional complex is greatly reduced. Published reports assert a role for USP4 in metastatic spread of lung cancer to the brain, mediated by its effects on the Wnt/β-catenin pathway. Potential functional compensation by USP15 must be evaluated before targeted therapies can be considered. Our studies will establish the extent and mechanism of such compensation.

A27 Stage I lung adenocarcinoma gene expression associated with aggressive histologic features for guiding precision surgery and therapy. J. Zhang[1], E. Burks[2], T. Sullivan[3], J. Sands[3], S. Regis[3], B. McKee[3], A. McKee[3], S. Zhang[1], H. Liu[1], G. Liu[1], S. Dubinett[4], A. Spira[5], J. Beane[1], K. Rieger-Christ[3], M. Lenburg[1]. [1]Boston University School of Medicine, Boston, MA, [2]Boston Medical Center, Boston, MA, [3]Lahey Hospital Medical Center, Burlington, MA, [4]David Geffen School of Medicine at UCLA, Los Angeles, CA, [5]Johnson and Johnson Innovation, Boston, MA.

Background: Stage I lung adenocarcinomas (LUADs) show heterogeneity in histologic patterns that correlate with malignant behavior. Solid, micropapillary, and cribriform patterns are associated with worse survival whereas lepidic (in situ) predominance has the best prognosis. In this study, we sought to characterize histologic pattern-specific gene expression in resected clinical stage I LUADs. We also aimed to train and validate a genomic biomarker predictive of histologic aggressive patterns with the ultimate goal of being able to impact surgical and therapeutic decision making for post-biopsy management.

Methods: A training cohort of 56 tumors from patients with stage I LUAD was included for pathologic annotation and whole-exome RNA sequencing. Histologic pattern subtyping in 5% increments including all diagnostic slides was performed. A single representative FFPE block was chosen for RNA sequencing. Negative binomial models were used to identify gene expression differences associated with percent solid, cribriform, or micropapillary histology, and EnrichR was used for pathway enrichment analysis. A random-forest classifier predicting aggressive histologic patterns was trained using 5-fold cross validation. An independent set of ≤2.0 cm clinical stage I LUAD was macrodissected into 32 paired components (lepidic + non-lepidic regions) and subjected to RNAseq. Six tumors were defined as low malignant potential (LMP: lepidic + acinar/papillary) and ten tumors were defined as overtly malignant potential (OMP: lepidic + solid/micropapillary/cribriform).

Results: In the training cohort, we identified 1,322 genes associated with tumor histologic composition (FDR q <0.05 and fold-change > 2). Genes whose expression differs with solid histology% were enriched for involvement in DNA replication, cell cycle regulation, and inflammation (FDR q<0.001). Genes associated with micropapillary% were enriched for involvement in tRNA-aminoacylation and decrease of T-cell activity (FDR q<0.001). The functional enrichment of genes associated with cribiform% was less informative. A gene expression classifier was trained to predict the presence of aggressive histology. We validated this classifier on a set of 16 tumor specimens from which we macrodissected and analyzed tissue from the most aggressive histologic pattern (AUC = 0.92). We also found that this classifier could differentiate between lepidic regions isolated from OMP and LMP tumors (AUC = 0.81).

Conclusion: We identified solid- , micropapillary- , and cribriform-specific gene expression among clinical stage I LUADs and developed a classifier predictive of aggressive histologic features using either lepidic (in situ) or nonlepidic components. This biomarker has the potential to predict histologic aggressiveness even from presurgical tumor biopsies where all histologic patterns may not be represented. Such a biomarker may be useful in guiding clinical decision making, including extent of surgical resection.

A28 Investigating antitumor T-cell responses using NINJA: An inducible genetic model for creating neoantigens. B. Fitzgerald, M. Damo, N. S. Joshi. Yale University School of Medicine, New Haven, CT.

Historically, attempts to generate inducible neoantigens in mouse models have been hindered by leaky expression of the antigen in the thymus, leading to central tolerance in developing CD8 and CD4 T cells. We have developed the iNversion INducible Joined neoAntigen (NINJA) model to resolve the existing problems of tolerance and leakiness using RNA splicing, DNA recombination, and three levels of regulation to control induction of neoantigen. Furthermore, this inducible model system is compatible with existing Cre-driven models of cancer, and we have generated a NINJA-antigen-inducible tumor cell line from a $Kras^{G12D}P53^{-/-}$ mouse lung tumor. Antigen expression in this model is temporally controlled via systemic drug delivery and generates responses in both transgenic and endogenous CD8 T cells. We will use this model to investigate specific T-cell responses to tumors and to assess how therapies such as checkpoint blockade impact T-cell response.

A29 Immune-suppressive microenvironment induced by increased Treg during EGFR-TKI mediated IP-10 and TGF-β. Sook-hee Hong[1], Nahyeon Kang[2], Okrane Kim[2], Seung Joon Kim[1]. [1]Seoul St. Mary's Hospital, The Catholic University of Korea, Seoul, Republic of Korea, [2]Cancer Research Institute, The Catholic University of Korea, Seoul, Republic of Korea.

Background: Studies on the immune microenvironment of EGFR mutant lung cancer have been limited. We analyzed the effect of immune microenvironments on the development of EGFR-TKI resistance in EGFR-mutated lung cancer.

Methods: The EGFR mutant lung cancer cell lines (HCC827 and H4006) were cocultured with activated PBMC for 72 hours with EGFR-TKI. Changes of cytokines/chemokines in the media, PD-1 expression of CD8+ T cells, regulatory T cells fraction, and transcriptome analysis of tumor cells were analyzed. We also performed immune profile analysis of fresh tissues of 21 surgically resected NSCLC (7 EGFR mutant and 14 EGFR wild) by multicolor FACS.

Results: IFN-γ, IL-6, VEGF, TGF- β1, and IP-10 were significantly increased after coculture but did not decrease after EGFR-TKI. PD-L1 expression on tumor cells increased after coculture (p = 0.08 in HCC827 and p = 0.09 in H4006) but did not decrease after coculture with activated PBMC and EGFR-TKI treatment (p = 0.36 in HCC827 and p = 0.45 in H4006). PD-1 expression of CD8 T cell cocultured with HCC827 or H4006 did not change; however, proportion of regulatory T cell

increased after coculture with HCC827 or H4006 (p=0.05 and p=0.08, respectively) and did not decrease during EGFR-TKI treatment. Proportion of regulatory T cell in cocultures with A549 or H1975 (erlotinib resistant cell line) did not change during coculture or EGFR-TKI treatment. Increase of IP-10 is mediated by IFN-γ in both EGFR mutant cell lines and PBMCs. The inhibition of IP-10 by si-RNA significantly decreased TGF-β1 expression and proportion of regulatory T cells in cocultured mutant EGFR lung cancer cell with EGFR-TKI treatment. Transcriptome analysis by RNA sequencing showed 1,747 gene sets were differentially expressed in EGFR-TKI treated EGFR mutant cell line cocultured with activated PBMC compared to EGFR-TKI treatment alone. Interferon gamma response pathway (NES 2.65, FDR q<0.1) was most significantly changed. Immune profile analysis of human EGFR mutant lung cancer showed marked heterogeneity in total lymphocyte infiltration, as low as 8.03% or as high as 44.7% of live cells. Among immune cells, proportion of CD4+/CD3+T cells in EGFR mutant groups was increased compared to EGFR wild group (62.7 ± 2.96 vs. 55.14 ± 5.1% among CD3+ T cells) and proportion of FOXP3+CD25+CD4+ Treg in EGFR mutant group tended to increase compared to EGFR wild group (1.352±0.4 vs. 0.74 ± 0.16%, p=0.256).

Conclusions: The increased regulator T cell by IP-10 and TGF-β is considered to be important in EGFR mutant NSCLC in immune-suppressive microenvironement and EGFR-TKI resistance.

A30 Tumor-infiltrating lymphocytes (TILs) found elevated in lung adenocarcinomas (LUAD) using automated digital pathology masks derived from deep-learning models. M. I. Jaber[1], L. Beziaeva[2], S. C. Benz[3], S. K. Reddy[2], S. Rabizadeh[3], C. W. Szeto[3]. [1]NantOmics, Culver City, CA, [2]NantHealth, Culver City, CA, [3]ImmunityBio, Santa Cruz, CA.

Background: Tumor mutation burden (TMB) is associated with increased response to anti-PD-1 therapy in non-small cell lung cancer (NSCLC) (Rizvi, 2015). Squamous cell carcinomas (LUSC) have higher average TMB than adenocarcinomas (LUAD) (Schumacher, Schreiber, 2015); however, meta-analyses show that in fact LUAD receive slightly more survival benefit from anti-PD1 therapy (Zhou, 2018). Here we explored whether lymphocyte distribution in the tumor microenvironment may give a rational explanation for this differential response to immuno-oncology (IO) agents.

Methods: 867 subtyped NSCLC high-resolution diagnostic whole-slide images were obtained from TCGA sources. Images were tiled into 100micron 2D color patches. To ensure subtypes were visually distinct at this scale, a LUAD/LUSC classifier was developed as follows: Samples were randomly split into 80% training and 20% testing samples. Cells were counted in each image patch and used to bin into 12 ranges of cell counts (0-10 cells per patch, 10-20, etc., up to >110 cells per patch). 2D color patches were transformed into 1D descriptive vectors using the *ResNet34* deep learning framework and used to train 12 separate support-vector machines (SVMs). An ensemble of these 12 SVMs was used to classify unseen samples. To detect tumor regions and lymphocyte infiltration, 2D color patches were used to train convolutional neural networks (*InceptionV3*) based on gold-standard masks generated with pathology assistance, then used to detect tumor and lymphocytes in all unseen patches. Patches that simultaneously classified as positive for tumor and lymphocyte area were considered evidence of TILs. Lymphocyte-positive patches immediately adjacent to tumor patches (i.e., lymphocytes within 100microns of tumor) were also analyzed.

Results: LUAD and LUSC were highly classifiable using this system, with a ROC AUC of 0.95 and precision of 0.95 in test samples. The total tumor tissue area was similar between samples classified as LUAD and LUSC (48.3%+/-1.1% vs. 46.5%+/-1.1%). Whole-slide lymphocyte level was similar although slightly lower in LUAD (9.9%+/-0.2% vs. 11.4%+/-0.2%). However, lymphocytes in LUAD samples were more likely to infiltrate tumor regions than those in LUSC (48.1%+/-1.2% vs. 42.7%+/-0.7%), and/or were immediately adjacent to tumor regions (78%+/-1.2% vs. 74.2%+/-0.9%). Lymphocyte levels were more bimodal in LUAD than LUSC, with 28.6% (vs. 22.9%) having very high TIL (>60%) despite having lower overall lymphocyte counts.

Conclusions: Despite lower overall TMB and lymphocyte levels, there exists a subset of LUAD samples with very high infiltrating lymphocyte counts, indicating a potentially anti-PD1-sensitive subpopulation. Further characterizing this subset and confirming differential IO response is warranted.

A31 A reservoir of tumor-specific CD8 T cells in lung cancer resides in the draining lymph node. K. Connolly, B. Fitzgerald, M. Nader, N. Joshi. Yale University, New Haven, CT.

This abstract is being presented as a short talk in the scientific program. A full abstract is printed in the Proffered Abstracts section (PR05) of the Conference Proceedings.

A32 Evaluation of the mutant KRAS-driven NSCLC tumor immune microenvironment using patient-derived cell line xenografts in a humanized mouse preclinical model for development of new immunotherapy approaches. H. Li, C. Han, H. Park, A. Zhang, Z. Liu, L. Girard, N. Sorrelle, R. A. Brekken, Y. Fu, J. D. Minna. UT Southwestern Medical Center, Dallas, TX.

Purpose of the Study: Tumor cell and tumor microenvironment (TME) features that influence the response to immune checkpoint blockade in lung cancer are incompletely defined. We wanted to develop preclinical models of lung cancer to explore the relationship of non-small cell lung cancer (NSCLC) molecular characteristics (oncogenotype such as mutant KRAS and LKB1/STK11, and mRNA expression profiles) to the human TME.

Experimental Procedures: We exploited the use of 8 (all KRAS mutant) molecularly characterized (whole-exome seq mutation and RNAseq analyses) patient-derived NSCLC lines grown as xenografts in vivo in humanized NSG-SGM3 mice in an effort to determine the effect of different NSCLCs on the immune landscape of lung cancer xenografts. The triple transgenic NSG-SGM3 (NSGS) mice express human IL3, GM-CSF, and SCF, which combine the features of the highly immunodeficient NOD scid gamma (NSG) mouse with cytokines that support the stable engraftment of human myeloid lineages and regulatory T-cell populations. Subcutaneous KRAS mutant-driven non-NSCLC xenografts grown in NSG-SGM3 mice "humanized" with CD34+ cord blood cells were subjected to immune landscape analysis through flow cytometry, multiplex immunohistochemistry, and cytokine analysis. The xenografts were also treated with various combinations of checkpoint inhibitors, radiation, and activation of the innate immune pathway with emricasan (pan caspase inhibitor).

Summary of New Data: Our results show: that the 8 KRAS mutant NSCLCs each were associated with a different spectrum of human TME; 3 of the 8 xenografts had only 0.1% of immune cell infiltrates while other xenografts had 20% immune cell infiltrates; that tumor mutation burden (TMB, absolute mutation calls 233 to 2,076) is not predictive of CD8+ T-cell infiltration in KRAS mutant-driven NSCLC xenografts. In NSCLC xenografts with high CD8+ T-cell infiltrate, the CD8+ cells in the TME were not activated, resulting in limited responses to PD-1/PD-L1 therapy. However, stimulation of the cGAS-STING innate immune pathway with emricasan followed by radiation (15 cGy) resulted in dramatic antitumor response.

Conclusions: NSCLC xenografts grown in "humanized" mice show great intertumor heterogeneity effects on the TME even within the KRAS mutant subgroup, and it is possible to demonstrate targeted therapy such as emricasan/radiation can lead to changes in improved antitumor responses.

A33 Phase I trial of in situ vaccination with autologous CCL21-modified dendritic cells (CCL21-DC) combined with pembrolizumab for advanced NSCLC. B. Liu, A. Lisberg, R. Salehi-Rad, J. M. Lee, L. M. Tran, K. Krysan, R. Li, R. J. Lim, C. Dumitras, Z. Jing, F. Abtin, R. D. Suh, S J. Genshaft, S. Oh, D. R. Aberle, L. E. Winter, S. Sharma, D. Elashoff, E. B. Garon, S. M. Dubinett. University of California Los Angeles, Los Angeles, CA.

Effective immunotherapy options are lacking for patients with advanced non-small cell lung cancer (NSCLC) who progress on a programmed cell death-(ligand)1 [PD-(L)1] inhibitor and for those who are epidermal growth factor receptor (EGFR) mutation- or anaplastic lymphoma kinase (ALK) rearrangement-positive after progression on tyrosine kinase inhibitor (TKI) therapy. One potential approach to improve immune checkpoint efficacy in these patient populations is to promote tumor-specific T-cell activation via in situ vaccination with chemokine gene-modified functional antigen-presenting cells (APCs), which take advantage of the full repertoire of tumor antigens and convert the tumor into a lymph node-like environment. The chemokine C-C motif chemokine ligand 21(CCL21) promotes colocalization of naive T cells and dendritic cells (DCs) to promote tumor antigen presentation and facilitate T-cell activation. Our preclinical studies and phase I trial of intratumoral (IT) administration of CCL21 gene-modified DC (CCL21-DC) revealed augmentation of tumoral CD8+ T-cell infiltration and systemic antitumor immunity. However, increased PD-L1 expression was observed in some patient tumors, suggesting that tumor-mediated impairment of T-cell function may be forestalling a more robust antitumor response. Similarly, improved

anti-PD-(L)1 efficacy may be possible with enhanced T-cell infiltration and augmented APC function following IT CCL21-DC. Therefore, we are conducting a phase I trial combining IT CCL21-DC with pembrolizumab in patients with advanced NSCLC with tumors accessible for IT injection, who are either (1) EGFR/ALK wild-type after progression on a PD-(L)1 inhibitor or (2) EGFR/ALK mutant after progression on TKI therapy. This is a phase I, single-institution, nonrandomized, dose-escalating, multicohort trial followed by dose expansion. A maximum of 24 patients (9-12 escalation + 12 expansion) with stage IV NSCLC will be evaluated. Three IT injections of autologous CCL21-DC (days 0, 21, 42) will be concurrently administered with pembrolizumab, followed by pembrolizumab once every 3 weeks for up to 1 year. Primary objective of dose escalation is safety and determination of maximum tolerated dose (MTD) of IT CCL21-DC when combined with pembrolizumab. Primary objective of dose expansion is objective response rate (ORR) of CCL21-DC at MTD combined with pembrolizumab. This trial, NCT03546361, is currently open for enrollment.

A34 Identification of Th1 epitopes in lung non-small cell lung cancer antigens to develop a multiantigen vaccine. L. Riolobos, E. Gad, M. L. Disis. University of Washington, Seattle, WA.

Non-small cell lung cancer (NSCLC) represents 85% of all lung cancer cases and it is highly smoking related. The goal of this project is to develop a vaccine for lung cancer prevention in current or past smokers by identifying immunogenic proteins in lung cancer that are able to induce a potent inflammatory Th1 response. Lung cancer has one of the highest mutation rates of all types of cancer, but driver mutations that could be targeted for a vaccine for lung cancer prevention are unknown. Gene expression profiling of bronchial biopsy specimens from smokers has shown that changes in gene expression in histologically normal epithelia can discern people with and without lung cancer. Many of these changes are proteins aberrantly upregulated, but not mutated. We have used quantitative mass-spectroscopy analysis to identify proteins overexpressed in NSCLC cell lines compared with normal lung epithelial cells. Five NSCLC cell lines (three squamous cell carcinoma and two adenocarcinoma) and two normal lung epithelial cell lines were included in the analysis. A total of 14,219 peptides, corresponding to 2,875 proteins, were identified. We selected for further analysis those proteins identified with >95% confidence and at least 3 peptides per protein and overexpressed in three or more NSCLC cell lines. We considered that a protein is overexpressed if [expression in the NSCLC cell line/ expression in the normal cell line]>1.5. A total of 154 antigens met our criteria. Candidate antigens were investigated by siRNA screening to identify those genes with a function in maintaining cell tumor growth. If a gene is required for tumor cell proliferation, knocking down the gene by siRNA should decrease cell survival and proliferation. We looked at both viability and apoptosis by caspase 3/7 activation after siRNA knockdown. We selected those antigens for which: [(mean of viability in NSCLC cell line) / (mean of viability in the normal lung cell line)] < 0.75 with a p-value of 0.1. We identified 14 candidates that are overexpressed in lung cancer and necessary for tumor cell survival. We have prioritized those proteins that have been previously described to play a role in lung cancer invasion, proliferation, metastasis, or survival. We selected 5 candidates to move forward: FKBP3, PARP1, RAN, S100A6, and SART3. An effective anticancer immune response needs to elicit a strong inflammatory Th1 response and avoid a Th2 response that promotes tumor tolerance. We used web-based modeling to predict epitopes that preferentially elicit a Th1 response and assessed the presence of Th1 and Th2 responses via IFN-g (Th1) and IL10 (Th2). Six to seven epitopes (15-20 mer peptides) per antigen were evaluated by IFN-g and IL10 ELISPOT. Th1 epitopes identified in NSCLC antigens are the base for a preventive vaccine for NSCLC. The efficacy of the multiantigen Th1 vaccine to prevent lung cancer is currently under evaluation in the NTCU-induced lung cancer mouse model.

A35 Dendritic cell in situ vaccination potentiates anti-PD-1 efficacy and induces immunoediting in a murine model of NSCLC. R. Salehi-Rad, R. Li, R. Lim, L. Tran, J. Abascal, S. Ong, B. Liu, S. Dubinett. University of California Los Angeles, Los Angeles, CA.

This abstract is being presented as a short talk in the scientific program. A full abstract is printed in the Proffered Abstracts section (PR07) of the Conference Proceedings.

A36 Patient-specific humanized PDX model for overcoming tumor resistance to immune checkpoint inhibitors in NSCLC patients. Ariel Sobarzo[1], Laila OptionaCl Roisman[2], Oleg Pikovsky[2], Lena Atlas[2], Petros Christopoulos[3], Holger Sültmann[4], Nir Peled[2]. [1]Ben-Gurion University of the Negev, Beer-Sheva, Israel, [2]Soroka University Medical Center, Beer-Sheva, Israel, [3]Thoraxklinik and National Center for Tumor

Diseases, Heidelberg University Hospital, Heidelberg, Germany, [4]German Center for Cancer Research (DKFZ), Heidelberg, Germany.

Background: Lung cancer is the most common cause of cancer-related mortality worldwide. Over the past few years, immune checkpoint inhibitors (ICI) have been shown to provide unprecedented clinical success in non-small cell lung cancer (NSCLC). However, ICI have some drawbacks, including initial and acquired resistance, which was observed after a complete response during and after previous ICI treatment. This relapse phenomenon was suggested to be associated with the state of the immune system and the tumor-immune response microenvironment interaction. The critical observation of cancer resistance or progression under ICI treatment suggests that a better and deeper understanding of the dynamic responses between the antitumor immune system and the tumor interaction, as it accrues in the patient setting, is therefore of utmost importance.

Methods: Using a patient-specific humanized patient-derived xerograph (PDX) (huMicX) model, we will study the coevolution between tumor and the immune system with and without ICI intervention. Comprehensive OMICS analysis on the proteomic, transcriptomic, and genomic levels will be performed on samples collected from human patients and the huMicX model.

Results: Sample biobank of whole blood and tumor tissues, and consensus protocols for peripheral HSC CD34+ isolation, are being established from NSCLC patients. Tumor tissue samples have been used to generate a PDX in mice model. Data from PDX models have demonstrated the feasibility of testing the activity of autologous transplanted lymphocytes against the patient's tumor in vivo with a clinical benefit in the same patient overcoming ICI resistance.

Conclusion: The huMicX model is designed to provide vital knowledge of the patient-specific tumor and immune system microenvironment, and the dynamic assessment of the mechanisms of ICI tumor resistance. This preclinical model is expected to present both treatment intervention and prognostic or predictable biomarkers, which will be exploited subsequently in actual clinical settings.

A37 N-803 plus nivolumab for advanced or metastatic non-small cell lung cancer: Update on phase II experience of combination PD1 blockade with an IL-15 superagonist. J. Wrangle[1], V. Velcheti[2], M. Patel[3], M. Sweiderska-syn[1], L. Macpherson[1], C. Coggins[1], C. Kreig[1], W. Redmond[4], A. Rock[5], J. Lee[5], M. Rubinstein[1]. [1]Medical University of South Carolina, Charleston, SC, [2]New York University, New York, NY, [3]University of Minnesota, Minneapolis, MN, [4]Earle A. Chiles Research Institute, Portland, OR, [5]ImmunityBio, Los Angeles, CA.

This abstract is being presented as a short talk in the scientific program. A full abstract is printed in the Proffered Abstracts section (PR12) of the Conference Proceedings.

A38 Gemcitabine improves suppressive immune microenvironment induced by long-term treatment with EGFR-TKIs: Implications for combination chemotherapy and immunotherapy. X. Wu[1], J. Tang[1], X. Liu[2], Q. Ma[1], P. Shu[1], Q. Deng[1], K. Li[1], B. Zhang[1], Y. Wang[1]. [1]Department of Thoracic Oncology, Cancer Center, West China Hospital, Sichuan University, Chengdu, Sichuan, China, [2]Department of Oncology, Sichuan Cancer Hospital, Chengdu, Sichuan, China.

Background: For patients harboring epidermal growth factor receptor (EGFR)-sensitive mutations, the use of EGFR tyrosine kinase inhibitors (EGFR-TKIs) has brought admirable survival. However, patients with EGFR mutation cannot benefit from anti-PD-1/PD-L1 alone as second-line therapy, from the analysis of results of immunotherapy clinical trials. In fact, immunotherapy with programmed cell death 1/ligand 1 (PD-1/PD-L1) checkpoint inhibitors is less effective in patients who previously received targeted therapy. For poor response to immune checkpoint inhibitors, one mechanism is suppressive immune microenvironment. However, the results of clinical trials, chemotherapy combined with pembrolizumab nivolumab, ipilimumab and atezolizumab, have mostly improved overall survival (OS) and progression-free survival (PFS) of non-small cell lung cancer (NSCLC) patients. The aims of this study were to determine whether gemcitabine or pemetrexed improves suppressive immune microenvironment induced by long-term treatment with EGFR-TKIs.

Methods: We adopted long-term use of EGFR-TKI models to investigate the responses of immune microenvironment to gemcitabine and pemetrexed. We analyzed the serum levels of IL-1β, IL-6, and IL-10 after chemotherapy.

Results: In our investigation, a significantly higher percentage of myeloid-derived suppressor cells (MDSCs) was detected in long-term erlotinib-treated mice. Compared with the pemetrexed for the long-term use of EGFR-TKI models, the level of MDSCs was consistently

reduced, CD8+ T cells, CD4+ T cells, and dendritic cells were elevated. Analysis of inflammatory factors in serum showed that gemcitabine decreased the levels of L-1β, IL-6, and IL-10.

Conclusion: These data suggested that gemcitabine could reverse MDSC-mediated immune suppression and modulate the tumor microenvironment, thereby improving the efficacy of immune-based therapies. The results indicated a combination therapy using chemotherapy and immunotherapy for patients with EGFR mutation or who acquired resistance to EGFR-TKIs. It was also suggested that the combination use of MDSC-scavenging drugs may enhance the efficacy of anti-PD-1 immunotherapy.

A39 Reactive cutaneous capillary endothelial proliferation caused by camrelizumab (SHR-1210) through activation of HIF-1α/VEGF signaling pathway. X. Wu[1], X. Zhang[2], P. Shu[1], Q. Ma[1], Y. Chen[1], D. Li[3], Y. Wang[1]. [1]Department of Thoracic Oncology, Cancer Center, West China Hospital, Sichuan University, Chengdu, Sichuan, China, [2]Department of Oncology, The Second Affiliated Hospital of Chongqing Medical University, Chongqing, China, [3]Precision Medicine Center, Precision Medicine Key Laboratory of Sichuan Province, West China Hospital, Sichuan University, Chengdu, Sichuan, China.

Background: Monoclonal anti-programmed cell death 1 (PD-1) antibodies are effective cancer therapeutics, but camrelizumab (SHR-1210) caused reactive cutaneous capillary endothelial proliferation (RCCEP) in patients. This symptom was not detected in the clinical trials of other PD-1/PD-L1 antibodies approved by the Food and Drug Administration (FDA). Therefore, it is of great significance to verify the phenomenon of camrelizumab (SHR-1210) to promote the proliferation of human blood vessels and to explore its possible mechanisms for the effective control of its side effects and the continuation of clinical trials.

Methods: We chose the cells mainly involved in the blood vessels, umbilical vein endothelial cells (HUVEC), as experimental object. Cholecystokinin c-terminal octapeptide (CCK-8) assay was used to detect the proliferation of HUVEC cells. Transwell cell migration and invasion assay were used to detect the cell migration ability; apoptosis detection by terminal-deoxynucleotidyl transferase-mediated nick end labeling (TUNEL) assay was used to detect the apoptosis; ELISA was used to detect the expression of VEGF and bFGF in the cell supernatants; Western blot test was used to detect HIF-1α, p44/42 (ERK1/2), p-p44/42 (p-Erk1/2), p38, p-p38, JNK, p-JNK, Akt, and p-Akt.

Results: The CCK-8 test suggested that 150 μg/ml and 200 μg/ml camrelizumab (SHR-1210) compared to the control group showed a significant increase in cell proliferation. We chose 150 μg/ml as the working concentration for follow-up experiments. Transwell cell migration and invasion assay suggested that the number of cell migration increased significantly in the camrelizumab (SHR-1210) treated group. The results of apoptosis detection by terminal-deoxynucleotidyl transferase-mediated nick end labeling (TUNEL) assay showed that there was no significant difference in the number of apoptotic cells and apoptosis index (AI) between the camrelizumab (SHR-1210) treated group and the control group. ELISA results showed that the concentration of VEGF in the supernatant of the camrelizumab (SHR-1210) treated group was significantly higher than that of the control group, but there was no significant difference in the concentration of bFGF. Western blot results indicated the expression of HIF-1α was significantly increased in the camrelizumab (SHR-1210)-treated group, and the expression of p-p44/42 (p-Erk1/2) and p-p38 was significantly increased, while p-JNK and p-Akt were not significantly increased.

Conclusion: Camrelizumab (SHR-1210) can promote proliferation and migration of HUVEC cells without inhibiting apoptosis. It can promote the expression of VEGF in HUVEC cells and promote the proliferation and migration of HUVEC cells through VEGF without promoting the expression of bFGF. By activating the HIF-1α/VEGF pathway and its upstream signal pathways ERK and p38MAPK, HUVEC cell proliferation was promoted instead of the JNK pathway and Akt-related pathway.

A40 Antioxidant, anti-inflammatory, and antiapoptotic potential of curcumin in benzo(a)pyrene (BaP)-induced lung injury in rats. Saleh Almatroodi, Arshad Rahmani. Qassim University, Buraydah, Qassim, Saudi Arabia.

Benzo(a)pyrene (BaP) is a well-known pollutant that directly induces inflammatory microenvironment in the lung. It also enhances oxidative stress and apoptosis and interferes with several other molecular pathways including cell death, survival, and proliferation that disturb normal homeostasis of the lung. Curcumin (Cur) has potent anti-inflammatory, antioxidant activity that defends cells from oxidative stress and cell death.

The objectives of the present study were to explore the protective effects of curcumin against long-term administration of BaP-induced disturbances in lungs of rats. Male rats were randomly divided into four groups: saline control, BaP only, BaP + Cur, and Cur only. Lung injury histopathology, electron microscopy, inflammatory cytokine release, antioxidant levels, apoptosis, and cell cycle were examined. Instillation of BaP significantly increased infiltration of inflammatory cells in alveolar space and inflammatory cytokine in blood. Histopathologic examination found BaP-induced pulmonary inflammatory changes were improved after administration of curcumin as evident by less infiltration of macrophages and neutrophils in alveolar space, less deposition of collagen, and edema. Furthermore, electron microscopy results also showed necrotic changes and broken cell membrane of Type II epithelial cell (T2E) of alveoli in BaP group, which was reduced after addition of curcumin treatment. In addition, we found BaP plus curcumin treatment effectively reduced inflammatory cytokines TNF-α and IL-6 in blood serum, but no significant changes were found in CRP levels. Moreover, the levels of tunnel staining and p53 expression were significantly increased by BaP, whereas these changes were noticeably modulated after curcumin treatment. Bap also interferes in normal cell cycle, which was markedly improved with curcumin treatment. Overall. these findings suggest that curcumin attenuates BaP-induced lung injury, probably through inhibiting inflammation, oxidative stress, and apoptosis in lung epithelial cells, and improving cell proliferation and antioxidants' level. Thus, curcumin may be an alternative therapy for improving the outcomes of benzo(a)pyrene-induced lung injury.

A41 EO1001: A first-in-class irreversible pan-ErbB inhibitor with excellent brain penetration. W. Shen[1], J. Bacha[1], S. Kanekal[1], N. Sankar[1], W. ZhenZhong[2], Y. Yoshida[3], T. Ozawa[3], T. Yao[3], A. Parsa[4], J. Raizer[4], S Cheng[4], A. Stegh[4], F. Giles[4], H. Pedersen[1], J. Sakaria[5], N. Butowski[3], C. James[4], D. Brown[1]. [1]Edison Oncology Holding Corp., Menlo Park, CA, [2]Jiangsu Kanion Pharmaceutical Co. Ltd., Lianyungang, China, [3]University of California San Francisco, San Francisco, CA, [4]Northwestern University, Feinberg School of Medicine, Chicago, IL, [5]Mayo Clinic, Rochester, MN.

Background: ErbB receptor tyrosine kinases EGFR (ErbB1), HER2 (ErbB2, neu), HER3 (ErbB3), and HER4 (ErbB4) are part of a complex network activating signaling pathways involved in cell growth and survival. Mutations causing errant ErbB activation are an oncodriver in many cancers including NSCLC. Inhibitors targeting ErbB mutations have transformed outcomes for patients; however, resistance to treatment develops rapidly. The various ErbB receptors have overlapping roles in oncogenesis and crosstalk between ErbB family members is associated with acquired resistance and metastases. For example, amplification of HER2 is a well-established mechanism of acquired resistance to EGFR-TKIs. The development of next-generation agents targeting multiple ErbB receptors has shown promise but has been limited by toxicity and poor brain penetration. Up to 80% of NSCLC patients will experience a brain lesion associated with their disease; treatment-resistant phenotypes metastasizing to the brain have become an important driver of morbidity and mortality and patients have limited therapeutic options. New agents are needed to address this important and growing unmet medical need. EO1001 is a first-in-class, oral, brain-penetrating, irreversible pan-ErbB inhibitor targeting ErbB1, ErbB2, and ErbB4 that is positioned for near-term entry into clinical development.

Methods: In vitro testing: EO1001 exhibits excellent and balanced equipotent activity against all three important ErbB receptors including EGFR, HER2, and HER4 with low nM activity (0.4 to 7.4 nM), with high specificity vs. off-target receptors. In vivo studies: Following oral administration, EO1001 treatment resulted in a statistically significant improvement in outcomes compared to positive and negative controls in erbB-positive mouse orthotopopic models including N87 (Her2+), H1975 (EGFR/T790M), GBM12 (EGFR+), GBM39 (EGVRvIII+). EO1001 rapidly enters the CNS at high concentrations relative to plasma and inhibits signaling downstream of mutant ErbB receptors in tumor tissue. Treatment with EO1001 was generally well tolerated with no gastrointestinal side effects observed at efficacious doses in mouse xenograft models.

PK and Toxicity Results: Preclinical pharmacokinetic and toxicology studies have been completed. EO-1001 exhibits a half-life of 16-20 hours in rodent models. Toxicities typical of the ErbB inhibitor class, including gastrointestinal effects, weight loss, and decreased activity, were observed at higher dose groups in both rodent and non-rodent species. Extrapolation to human dosing suggests an attractive therapeutic window in comparison to other agents in the class.

Conclusion and Next Steps: EO1001 has the potential to be a best-in-class CNS-penetrating pan-ErbB inhibitor amenable for use as a single agent and in combination regimens. First-in-man clinical testing with EO1001 is planned. Continued characterization of EO1001 activity against specific ErbB mutations will be undertaken in parallel.

B01 Active YAP as a functional marker of drug-tolerant persister cells in EGFR-mutant and ALK fusion-positive NSCLC. F. Haderk[1], C. Fernández-Méndez[2], K. N. Shah[1], W. Wu[1], J. Guan[1], J. Rotow[3], D. Allegakoen[1], V. Olivas[1], S. Bandyopadhyay[1], C. Kuo[4], T. Bivona[1]. [1]University of California San Francisco, San Francisco, CA, [2]IIBm-UAM, Madrid, Spain, [3]Dana-Farber Cancer Institute, Boston, MA, [4]Stanford University, Stanford, CA.

Targeted therapies against clinically actionable oncogenic drivers in lung adenocarcinoma have significantly improved survival of cancer patients, but durable responses are limited due to the emergence of drug resistance. Resistance development is often characterized by the retention of a small subpopulation of cancer cells under drug treatment and their evolution from non-/low-proliferative residual disease to an aggressively growing resistant tumor. Most importantly, drug-tolerant persister cells have been identified as a reservoir for a multitude of drug resistance mechanisms and thus, their characterization and the development of rational combinatorial treatment may delay or prevent resistance development and improve treatment outcome for cancer patients. Using a multitude of in vitro models such as cell culture models and patient-derived organoids, we characterized signaling and transcriptional changes in drug-tolerant persisters. We identified YAP nuclear relocalization and its increased transcriptional activity as a key marker of persisters derived from EGFR-mutant and EML4-ALK fusion-positive specimen under third-generation TKI treatment. Image analysis of cells genetically engineered via CRISPR-Cas9 to express endogenously labeled YAP-mNeonGreen validated these results. Moreover, we were able to prove the functional relevance of YAP activation in drug persistence by overexpressing active mutants of YAP that are lacking inhibitory Hippo phosphorylation sites. The latter resulted in increased nuclear levels and transcriptional activity of YAP and mediated significantly reduced cell death under high-dose drug treatment in different cell line models. Using RNA sequencing, we show a clear evolutionary path from drug-sensitive parental cells to drug-tolerant persisters and long-term derived drug-acquired resistant cells. We are currently profiling vulnerabilities of drug-tolerant EGFR-mutant and EML4-ALK fusion persisters using genetic and pharmacologic approaches. In conclusion, YAP activation is a functional marker of EGFR-mutant and EML4-ALK fusion persisters derived under high-dose drug treatment with third-generation TKIs. Targeting YAP activation either on the level of upstream signaling input, its relocalization between cytoplasm and nucleus, or its action as transcriptional coactivator may represent a promising combinatorial treatment approach to limit resistance development and improve patient survival in lung adenocarcinoma.

B02 The GSK3 signaling axis regulates adaptive glutamine metabolism in lung squamous cell carcinoma. M. Momcilovic[1], J. T. Lee[1], D. Braas[1], T. G. Graeber[1], F. Parlati[2], S. Demo[2], R. Li[1], M. Gricowski[3], R. Shuman[3], J. Ibarra[3], D. Fridman[3], M. St.John[1], N. Bernthal[1], N. Federman[1], J. Yanagawa[1], S. M. Dubinett[1], S. Sadeghi[1], H. R. Christofk[1], D. B. Shackelford[1]. [1]University of California Los Angeles, Los Angeles, CA, [2]Calithera Biosciences, San Francisco, CA, [3]Memorial Care Health, Long Beach, CA.

Altered metabolism is known to generally contribute to cancer growth, forming the conceptual basis for development of metabolic therapies as cancer treatments. However, the specific metabolic characteristics of individual cancer types in vivo are still largely unknown, limiting the translatability of metabolic therapies in the clinic. In this study we performed in vivo metabolic profiling and molecular analysis of lung squamous cell carcinoma (SCC) using both positron emission tomography and mass spectrometry. We identify a metabolic signature in this subset of lung tumors characterized by a reliance on both glucose and glutamine. Lung SCC adapts to chronic mTOR inhibition and suppression of glycolysis through the GSK3α/β signaling pathway that upregulates glutaminolysis. Phospho-GSK3α/β protein levels are predictive of response to single-therapy mTOR inhibition while combinatorial treatment with the glutaminase inhibitor CB-839 effectively overcomes therapy resistance. Lastly, we identified a conserved metabolic signature in a broad spectrum of hypermetabolic human tumors that is predictive of patient outcome and response to combined metabolic therapies targeting mTOR and glutaminase. We therefore propose a new treatment paradigm for patients with lung SCC involving the use of a metabolic signature as a biomarker to select patients who will benefit from combined therapies targeting mTOR and glutaminase.

B03 JNJ-61186372, an Fc effector enhanced EGFR/cMet bispecific antibody, induces EGFR/cMet downmodulation and efficacy through monocyte and macrophage trogocytosis. S. Vijayaraghavan, L. Lipfert, K. Chevalier, B. Bushey, B. Henley, R. Lenhart, J. Sendecki, M. Beqiri, H. J. Millar, K. Packman, M. V. Lorenzi, S. Laquerre, S. L. Moores. Janssen Research & Development, Spring House, PA.

Small-molecule tyrosine kinase inhibitors (TKIs) have become standard of care in EGFR-mutated NSCLC, but acquired resistance invariably develops due to new mutations in EGFR and activation of compensatory pathways such as cMet. JNJ-61186372 (JNJ-372) is an anti-EGFR and cMet bispecific low-fucose antibody (huIgG1) with enhanced Fc function designed to target tumors with activated EGFR and cMet signaling through a novel mechanism of action. An ongoing first-in-human study to assess the safety and efficacy of JNJ-372 in patients with advanced, treatment-refractory NSCLC revealed JNJ-372 to have clinical activity in patients with diverse EGFR-mutated NSCLC, including tumors with EGFR mutations (Exon20, T790M, C797S) resistant to TKIs and those resistant due to *MET* amplification. Despite observing potent antitumor activity of JNJ-372 in EGFR mutant xenograft models, only modest antiproliferative effects were observed in NSCLC cell lines in vitro. We also found that the Fc inactive version (IgG2sigma) of the EGFR/cMet antibody was significantly impaired in its ability to inhibit tumor growth in mice compared to the Fc enhanced JNJ-372. The IgG2sigma variant also reduced the ability of the bispecific antibody to mediate downregulation of EGFR and cMet signaling. These observations suggested that the interaction of the Fc domain of the antibody with the Fcgamma receptors on innate immune cells may play a crucial role in the mechanism of action of JNJ-372. We performed a comprehensive assessment of the Fc effector functions of JNJ-372, including effects on EGFR and cMet levels, downstream signal transduction, and role in mediating antitumor activity. Using cancer cell lines in vitro, the addition of isolated human immune cells (PBMCs) significantly enhanced JNJ-372-mediated EGFR and cMet downregulation, and dose-dependent tumor cell killing. Through depletion or enrichment of specific immune cell types, we demonstrated that monocytes and/or macrophages are necessary and sufficient for JNJ-372 Fc interaction-mediated EGFR/cMet downmodulation and that macrophages are required for in vivo efficacy. Finally, through imaging studies tracking labeled JNJ-372, we visualized monocyte/macrophage-mediated trogocytosis. Collectively, these data demonstrate a novel Fc-dependent mechanism of action of JNJ-372 and support the continued clinical development in patients with aberrant EGFR and cMet signaling.

B04 Activity of larotrectinib in tropomyosin receptor kinase fusion lung cancer. A. F. Farago[1], S. Kummar[2], V. Moreno[3], J. Patel[4], U. Lassen[5], L. Rosen[6], B. H. Childs[7], D. M. Hyman[8], A. Drilon[8]. [1]Massachusetts General Hospital Cancer Center, Boston, MA, [2]Stanford Cancer Institute , Stanford, CA, [3]START MADRID-FJD, Hospital Fundación Jiménez Díaz, Madrid, Spain, [4]University of Chicago, Chicago, IL, [5]Department of Oncology, Rigshospitalet, Copenhagen, Denmark, [6]University of California Los Angeles, Los Angeles, CA, [7]Bayer HealthCare Pharmaceuticals, Whippany, NJ, [8]Memorial Sloan Kettering Cancer Center, New York, NY.

Background: Tropomyosin receptor kinase (TRK) fusions involving neurotrophic receptor tyrosine kinase (*NTRK)1*, *NTRK2*, and *NTRK3* genes occur in a range of tumor types. Larotrectinib, the first FDA-approved highly selective TRK inhibitor, has demonstrated an overall response rate (ORR) of 75% by independent central review across a broad spectrum of tumors that harbor *NTRK* gene fusions (Drilon et al., NEJM 2018;378:731–9). Here we report updated data on the lung cancer patients who have been treated with larotrectinib.

Methods: Patients with non-small cell lung cancer (NSCLC) from two clinical trials (NCT02122913 and NCT02576431) with TRK fusion cancer were included in this analysis. Larotrectinib (100 mg, twice daily) was administered on a continuous 28-day schedule until withdrawal, unacceptable toxicity, or disease progression. Here we report responses assessed by investigator (INV) per RECIST v1.1.

Results: As of February 19, 2019, 12 patients with metastatic lung adenocarcinoma were enrolled. Median age was 49 years (range 25–76). Nine patients had fusions involving *NTRK1* and diverse fusion partners: *TPM3* (n=2), *SQSTM1* (n=1), *IRF2BP2* (n=2), *TPR* (n=1), *CD74* (n=1), and *EPS15* (n=2). Three patients had fusions involving NTRK3 (fusion partner: *SQSTM1* [n=2] and *ETV6* [n=1]). Eleven patients had prior systemic therapy (six patients had three or more prior therapies) with best responses on last prior therapy being one partial response, four with stable disease, three progressive disease, and four unknown, unevaluable, or not applicable. All 12 patients were evaluated for response to larotrectinib as per INV assessment. ORR was reported in nine patients (75%) with one complete response, and eight partial responses with one partial response pending confirmation. The median time to response was 1.8 months. There were three patients with stable disease. Six of the 12 patients had brain metastases at the time of study enrollment, and the ORR in those six patients was 67%. The overall duration of response by INV ranged from 3.9+ months to 25.9+ months; the median duration of response not reached. One patient continued receiving treatment post-progression. Two patients discontinued treatment due to

disease progression and one discontinued due to disease progression in nontarget lesion. Larotrectinib was well tolerated, with treatment-related adverse events being predominantly grade 1–2.

Conclusions: Larotrectinib is highly active in patients with advanced lung cancer harboring NTRK gene fusions, including those with central nervous system metastases, with a favorable safety profile. These results support the use of larotrectinib in TRK fusion lung cancer.

B05 Identifying SCLC vulnerabilities using phenotypic chemical screens. J. Povedano, R. Rallabandi, D. Nijhawan, J. de Brabander, D. McFadden. University of Texas Southwestern Medical Center, Dallas, TX.

Small-cell lung carcinoma (SCLC) is an aggressive neuroendocrine cancer in which few actionable mutations have been uncovered in the last 30 years. With the goal of identifying chemically tractable proteins essential for SCLC viability, our lab has performed a phenotypic high-throughput small-molecule screen (HTS) in collaboration with the UTSW HTS Core Facility using a library of 200,000 drug-like compounds. We used a SCLC cancer cell line derived from a p53; Rb1 genetically engineered mouse model that recapitulates cardinal features of the human disease. By counter-screening against a panel of murine cancer cell lines (NSCLC, papillary thyroid cancer, and rhabdomyosarcoma), we identified 51 SCLC-selective toxins exhibiting at least 5-fold selectivity for SCLC cancer cells compared to the panel of non-SCLC cell lines. We hypothesized that identifying the target of these molecules will allow the discovery of important vulnerabilities for SCLC. To uncover the mechanism of action of these 51 SCLC-selective toxins, we are using two orthogonal approaches. One strategy uses forward genetics to identify compound resistant alleles that impair compound-target interaction. We recently demonstrated that engineering mismatch repair (MMR) deficiency into murine SCLC cancer cells (using CRISPR/Cas9 to silence *Msh2*) led to hypermutation and enabled the acquisition of compound resistant alleles for three anticancer compounds with known mechanisms of action. We used these cells to identify compound resistant alleles that co-occur in multiple resistant clones that emerge following selections in cell culture of these anticancer compounds. We are also using medicinal chemistry as another approach to elucidate the mechanism of action of the SCLC-selective toxins. In collaboration with the De Brabander lab, three from the 51 SCLC-selective toxins were chosen for suitability for medicinal chemistry efforts due to their high selectivity (over 10-fold). We are performing structure-activity relationship studies to optimize potency and selectivity as well as target-ID studies. We also developed analogs harboring cross-linkable moieties with the goal of being able to efficiently enrich candidate proteins that can be identified by mass spectrometry.

B06 Time-resolved RNA-seq identifies transient gene expression changes following initial chemotherapy challenge in small-cell lung cancer. D. W Shia, V. Vuong, E. Pecora, N. Balanis, P. Vijayaraj, C. Sen, C. J Aros, T. Rickabaugh, T. Graeber, B. Gomperts. University of California Los Angeles, Los Angeles, CA.

Small-cell lung cancer (SCLC) comprises about 15% of all lung cancer and exhibits a remarkably aggressive clinical course, with early metastasis, rapid development of chemoresistance, and an overall survival of 6%. While standard-of-care combination chemotherapy with platinum-based agents and etoposide elicits dramatic responses following initial treatment, chemoresistant disease develops rapidly and contributes to the poor mortality rate in this disease. Transcriptional changes and underlying epigenetic changes have increasingly been recognized in the development of chemoresistance across different cancer types and in response to a variety of neoplastic agents. Indeed, a notable study in SCLC identified a role of the enhancer of zeste homolog 2 (EZH2) histone-lysine methyltransferase in mediating a chemoresistant phenotype through silencing of the *SLFN11* gene product, a factor implicated in DNA damage repair deficiency. Furthermore, transient gene expression changes in survival cell fractions following chemotherapy have been demonstrated to contribute to disease relapse and can potentially be targeted. Given the exceptional initial response rates SCLC has to cisplatin and etoposide, we endeavored to define molecular changes that occur in surviving cell fractions following initial chemotherapy challenge to refine our understanding of SCLC relapse biology and identify candidate factors. We initially identified optimal dosing schemes across a panel of SCLC cell lines and quantified cell number and proliferation, establishing seven to ten days as a time window for maximal cytoreduction following chemotherapy in vitro. We then performed transcriptional profiling via RNA-sequencing on cell lines treated with either single-agent cisplatin or combination cisplatin + etoposide across a 24-day time course and utilized principal component analysis to identify genes whose expression exhibits transient expression patterns across the time course. Using gene set enrichment analysis, we confirmed fidelity of our dataset by

identification of expected transiently downregulated genes involved in ribosomal biogenesis and concordantly upregulated genes involved in xenobiotic response and DNA damage. Consistently, between both single-agent cisplatin and combination treatment time courses, we identified a significant transient upregulation of a suite of transcription factors. Importantly, we observed a 10- to 30-fold upregulation of these factors compared to baseline that is transient and peaks at timepoints with lowest absolute viable cell number. Current work is focused on determining the sufficiency and necessity of these factors in the progression of SCLC following initial chemotherapy.

B07 Mechanisms of alectinib resistance in a leptomeningeal carcinomatosis of EML4-ALK lung cancer and its circumvention by EGR-TKIs. S. Yano, S. Arai, K. Fukuda, S. Takeuchi. Kanazawa University, Kanazawa, Japan.

Central nervous system (CNS) metastasis, such as brain metastasis and leptomeningeal carcinomatosis (LMC), occurs in 20–40% of all patients with cancer. Anaplastic lymphoma kinase (ALK) is a clinically validated drug target, and ALK rearrangements are found in approximately 3-5% of non-small cell lung cancer (NSCLC). ALK tyrosine kinase inhibitor (TKI) shows dramatic clinical efficacy in ALK-rearranged NSCLC patients, and the second-generation ALK-TKI alectinib is effective against CNS metastasis of ALK-rearranged NSCLC. However, the patients with ALK-rearrangement acquire resistance to alectinib over time and develop recurrent LMC metastasis. This study aimed to clarify the mechanism of resistance to alectinib in LMC and seek a novel therapeutic strategy. Alectinib-resistant cell line (A925L/AR) was established by continuous treatment with alectinib in the LMC mouse model inoculated with the alectinib-sensitive human lung cancer cell line, A925LPE3, which harbors the EML4-ALK gene fusion. The tumor level was measured by in vivo imaging system. To clarify the mechanism of alectinib resistance, tumor cell culture supernatants, patient cerebrospinal fluid (CSF), and patient serum were measured using ELISA kits for EGFR ligands. A925L/AR cells were moderately resistant to various ALK-TKIs, such as alectinib, crizotinib, ceritinib, and lorlatinib, compared with parental cells in vitro. A925L/AR cells acquired resistance through epidermal growth factor receptor (EGFR) activation due to overexpression of its ligand, amphiregulin, via inhibited expression of microRNA 449-a. EGFR-TKIs and anti-EGFR antibodies resensitized A925L/AR cells to alectinib in vitro. In the LMC model with A925L/AR cells, combined treatment with alectinib and an EGFR-TKI, such as erlotinib and osimertinib, successfully controlled LMC progression. Imaging mass spectrometry showed accumulation of EGFR-TKIs in the tumor lesions. Moreover, notably high amphiregulin levels were detected in the cerebrospinal fluid from ALK-rearranged NSCLC patients with alectinib-resistant LMC compared with those in EGFR-mutated NSCLC patients with EGFR-TKI-resistant LMC or patients without LMC. We demonstrated that EML4-ALK lung cancer cells acquired moderate resistance to alectinib in the leptomeningeal space due to amphiregulin-triggered EGFR activation. Moreover, combined use of alectinib and EGFR-TKIs, including the third-generation inhibitor osimertinib, could overcome resistance in the LMC model. Our findings may provide rationale for clinical trials to investigate the effects of novel therapies dual-targeting ALK and EGFR in ALK-rearranged NSCLC with alectinib-resistant LMC.

B08 Impact of concurrent *STK11* loss and *c-MYC* amplification in metastatic non-small cell lung cancer (NSCLC). S. Menon[1], C. Ellis[2], S. Poudel[3], J. Johnson[4], A. Szabo[1], B. George[1], W. K. Kelly[4], S. Grant[5], J. McPherson[6], M. Cristofanilli[7], C. Hoimes[8], M. Gutierrez[9], J. Doudement[10], L. Chan[10], G. Singal[10], B. Alexander[10], V. Miller[10], D. Sohal[11]. [1]Medical College of Wisconsin, Milwaukee, WI, [2]UNC Chapel Hill, Chapel Hill, NC, [3]Cleveland Clinic, Cleveland, OH, [4]Thomas Jefferson University, Philadelphia, PA, [5]Wake Forest, Winston-Salem, NC, [6]University of California Davis, Sacramento, CA, [7]Northwestern University, Chicago, IL, [8]Seidman University Hospitals, Cleveland, OH, [9]Regional Cancer Care Associates, Hackensack, NJ, [10]Foundation Medicine, Boston, MA, [11]University of Cincinnati, Cincinnati, OH.

Introduction: Despite significant therapeutic advances, clinical outcome remains poor in most patients (pts) with NSCLC, due at least in part to their genotype. *STK11* is a master kinase that controls cellular metabolism, while *c-MYC* is an oncogene altered in many cancers promoting proliferation. Preclinical data (PMID:24793789) suggest that *c-MYC* amplification in the setting of *STK11* loss can lead to unchecked growth of cancer cells. We anecdotally observed rapid progression, primary treatment refractoriness, and dramatic clinical decline in several pts with metastatic NSCLC (mNSCLC) with concurrent *STK11* loss and *c-MYC* amplification. Hence, we investigated the incidence and the prognostic impact of these biomarkers in mNSCLC.

Methods: This study was performed through the Precision Medicine Exchange Consortium (PMEC), a consortium of 10 US academic medical centers that

share clinically annotated genomic data under a central IRB-approved protocol. The PMEC database (PMEC-DB) was queried for NSCLC pts with either *STK11* loss (cohort A), *c-MYC* amplification (cohort B), or both (cohort C). Comprehensive genomic profiling (CGP) was performed on tumor tissue utilizing the Foundation One 315 gene assay. Demographic and disease characteristics were analyzed. Survival curves were estimated using the Kaplan-Meier method.

Results: Among the 1,952 pts with NSCLC in the PMEC-DB, 396 pts met the inclusion criteria with 246 (62%), 103 (26%), and 47 (11.8%) pts in cohorts A, B, and C, respectively. Median TMB for the entire study set was 8.7; there was no statistically significant difference between the 3 cohorts (p = 0.12). KRAS mutations were detected more frequently in cohort A compared to cohorts B and C (58 % vs. 18% vs 38%; p<0.0001). Clinical outcome data were available in 99 (25%) pts and were distributed among cohorts A, B, and C, in similar proportion to the overall study set with 60, 24, and 15 pts, respectively. Cohort C was associated with a nonadenocarcinoma histology compared to cohorts A and B (53.3%, 16.7%, and 33.3%, respectively, p=0.011). Nonadenocarcinoma subtypes in Cohort C were NSCLC NOS 33.3%, squamous 6.7%, and large cell neuroendocrine 13.3%. There was no difference in median overall survival (mOS) between cohorts A, B, and C (10 months, 17 months, and 11 months respectively, p =0.68).

Conclusion: Concurrent *STK11* loss and *c-MYC* amplification in NSCLC is uncommon but had no impact on survival in a limited patient set. This study underscores the importance of large-scale, clinically annotated genomic data sharing initiatives in systematically exploring the clinical relevance of rare genomic alterations.

B09 The CANOPY program: Three phase 3 studies evaluating canakinumab in patients with non-small cell lung cancer (NSCLC). E. B. Garon[1], A. Ardizzoni[2], F. Barlesi[3], B. C. Cho[4], G. de Castro[5], E. Felip[6], Y. Goto[7], A. Greystoke[8], S. Lu[9], D. W.-T. Lim[10], M. Reck[11], B. J. Solomon[12], D. R. Spigel[13], D. S. W. Tan[10], M. Thomas[14], J. C.-H. Yang[15], B. E. Johnson[16]. [1]David Geffen School of Medicine at UCLA/TRIO-US Network, Los Angeles, CA, [2]S. Orsola-Malpighi University Polyclinic, Bologna, Italy, [3]Aix-Marseille University, Marseille, France, [4]Yonsei University College of Medicine, Seoul, Republic of Korea, [5]Instituto do Câncer do Estado de São Paulo, São Paulo, Brazil, [6]Vall d'Hebron Institute of Oncology, Barcelona, Spain, [7]National Cancer Center Hospital, Department of Thoracic Oncology, Tokyo, Japan, [8]Newcastle upon Tyne Hospitals, Newcastle, United Kingdom, [9]Shanghai Chest Hospital, Jiaotong University, Shanghai, China, [10]National Cancer Centre Singapore, Singapore, Singapore, [11]LungenClinic, Airway Research Center North (ARCN), German Center for Lung Research (DZL), Grosshansdorf, Germany, [12]Peter MacCallum Cancer Centre, Melbourne, VIC, Australia, [13]Sarah Cannon Research Institute, Nashville, TN, [14]Internistische Onkologie der Thoraxtumoren, Thoraxklinik im Universitätsklinikum Heidelberg, Translational Lung Research Center Heidelberg (TLRC H), Member of the German Center for Lung Research (DZL), Heidelberg, Germany, [15]Graduate Institute of Oncology, National Taiwan University College of Medicine, Taipei, Taiwan, [16]Dana-Farber Cancer Institute, Boston, MA.

Background: Canakinumab (CANA) is a selective IL-1β inhibitor that aims to target tumor-promoting inflammation to reduce immune suppression. In the CANTOS study, CANA treatment was associated with reduced lung cancer incidence and mortality in patients (pts) with stable post-myocardial infarction who had elevated high-sensitivity C-reactive protein levels, thus providing a rationale to investigate its possible therapeutic role in lung cancer.

Methods: CANOPY-A, CANOPY-1, and CANOPY-2 are phase III, multicenter, randomized, double-blind, placebo-controlled studies. In CANOPY-A, pts (~1,500) with stages IIA–IIIA and IIIB (T>5 cm N2), any histology, completely resected (R0) NSCLC, who received cisplatin-based chemotherapy (CTx), will be enrolled and randomized 1:1 to receive either CANA (200 mg Q3W SC) or placebo (Q3W SC) for 18 cycles. As of Oct 8, 2019, there are 278 study locations per clinicaltrials.gov. The primary endpoint will be disease-free survival. Key secondary endpoint will be overall survival (OS). CANOPY-1 and CANOPY-2 will each consist of part 1 (open-label, safety run-in) and part 2 (randomized, placebo-controlled; efficacy and safety evaluation). Eligible pts should have ECOG PS ≤1 and no *EGFR* sensitizing mutations and/or *ALK* rearrangements. In CANOPY-1, pts with previously untreated stage IIIB/IIIC–IV NSCLC and known PD-L1 status (part 2 only) will be enrolled. Part 1 will consist of 3 cohorts of ~9 pts each (based on different platinum-CTx) to confirm the recommended phase 3 regimen (RP3R) for CANA. Pts will be treated with full doses of CTx plus pembrolizumab plus CANA. Enrollment and safety observation period for part 1 is complete. In part 2, pts (~600) will be randomized (1:1) to receive CANA (200 mg Q3W SC) or placebo + pembrolizumab + platinum-doublet CTx for 4 cycles,

followed by maintenance until progressive disease. As of Oct 15, there are 129 study locations per clinicaltrials.gov. In CANOPY-2, pts with stage IIIB–IV NSCLC, who received prior PD-(L)1 inhibitor therapy and platinum-based CTx, and no PD-(L)1 selection, will be enrolled. Part 1 will enroll ~9 pts to confirm the RP3R of CANA. Pts will be treated with full doses of CANA 200 mg SC + docetaxel 75 mg/m^2 i.v. on day 1 of each 21-day cycle. Enrollment to part 1 of the study is complete. In part 2, pts (~226) will be enrolled and randomized 1:1 to receive CANA (200 mg Q3W SC) or placebo + docetaxel. As of Oct 23, there are 85 study locations per clinicaltrials.gov. In part 1 (both studies), the primary endpoint is the incidence of dose limiting toxicities in the first 42 days of treatment. In part 2, the primary endpoints are progression-free survival (PFS) and OS in CANOPY-1, and OS in CANOPY-2. Common secondary endpoints (both studies) include overall response rate, disease control rate, time to response, duration of response, PFS (CANOPY-2), pharmacokinetics, safety, patient-reported outcomes, and immunogenicity. All three studies (CANOPY-A, CANOPY-1, and CANOPY-2) are currently recruiting.

B10 Prevalence of EGFR mutation among Vietnamese non-small cell lung cancer: A preliminary study. Tu Van Dao[1], Khac-Dung Nguyen[1], Oanh Thi Bui[1], Quang Ngoc Nguyen[2], Linh Dieu Vuong[2]. [1]Cancer Research and Clinical Trial Center, National Institute for Cancer Control, National Cancer Hospital, Hanoi, Vietnam, [2]Pathology and Molecular Biology Center, National Cancer Hospital, Hanoi, Vietnam.

Aims: To investigate the distribution of epidermal growth factor receptor (EGFR) mutations, and explore any relationships with characteristics of non-small cell lung cancer (NSCLC) patients.

Materials and Methods: EGFR mutations were assessed by Scorpions and ARMS technologies (therascreen® EGFR RGQ PCR Kit - Qiagen) in randomized sample block of 200 NSCLC patients from Vietnam National Cancer Hospital. Relationships between EGFR mutation and patient characteristics were analyzed by R statistical software.

Results: The EGFR mutation rate was 41% (83/200); 19-del and L858R mutations occurred predominantly, accounting for 55.4% and 27.2%, respectively, in mutated cases. Moreover, 3.5% patients were found to carry double mutations. EGFR mutations occurred more frequently in women (75%) than in men (27.1%) (P<0.001). Mean ages of patient with mutation and without mutation were 56.51 (±8.86) and 58.83 years (±9.05), respectively (p=0.073). Gender distribution was significantly different between the 2 groups of mutation and no mutation (p<0.001). In EGFR mutation group, 98.8% of them possessed the Vietnamese health insurance and 9.6% of them which their first diagnosis had no relation with lung carcinoma.

Conclusions: The EGFR mutation rate was 41% in NSCLCs in Vietnam, so that about 40% of patients might benefit from targeted therapies. Further studies are required to have a comprehensive understanding about the other clinical characteristics and EGFR mutation in Vietnamese patients.

B11 Accurate detection of *METex14* mutations in non-small cell lung cancer (NSCLC) with comprehensive genomic sequencing: Results from the GEOMETRY mono-1 study. R. S. Heist[1], E. B. Garon[2], D. S. W. Tan[3], H. J. M. Groen[4], T. Seto[5], E. F Smit[6], N. Nwana[7], L. Fairchild[8], A. Balbin[8], M. Yan[8], I. Wang[7], M. Giovannini[7], B. Sankaran[8], J. Wolf[9]. [1]Massachusetts General Hospital, Boston, MA, [2]David Geffen School of Medicine at UCLA, Los Angeles, CA, [3]National Cancer Centre Singapore, Singapore, Singapore, [4]University of Groningen and University Medical Center Groningen, Groningen, Netherlands, [5]National Hospital Organization Kyushu Cancer Center, Fukuoka, Japan, [6]Netherlands Cancer Institute, Amsterdam, Netherlands, [7]Novartis Pharmaceuticals Corporation, East Hanover, NJ, [8]Novartis Institutes for Biomedical Research, Cambridge, MA, [9]Center for Integrated Oncology, University Hospital Cologne, Cologne, Germany.

Background: *MET* exon 14 skipping mutations (*METex14*) occur in 3–4% of patients (pts) with NSCLC. Accurate detection of the genomic variants that result in *METex14* in *MET*-driven tumors could facilitate timely intervention with selective MET inhibitors (METi) and improve clinical outcomes. Different detection assays for *METex14* using various platforms have yielded mixed results across studies. It is imperative to utilize reliable and validated molecular assays to identify pts to be treated with METi. RNA-based detection of *METex14* is considered the gold standard, since this assay measures the direct result of deletion of exon 14 event regardless of underlying genomic events. DNA-based next-generation sequencing (NGS) must detect genomic alterations within *MET* exon 14 and adjacent intronic regions that alter a splicing site or delete the whole *MET* exon 14.

Methods: The GEOMETRY mono-1 study evaluated the efficacy and safety of capmatinib in pts with *EGFR-wt*, *ALK-neg*, NSCLC harboring *METex14*. This retrospective analysis compared DNA-based NGS with RNA-based RT-PCR in detecting *METex14* in the GEOMETRY mono-1 study. Eligible *METex14*-mutated pts confirmed by RT-PCR qualitative assay using RNA extracted from baseline formalin-fixed, paraffin-dipped (FFPE) tissue samples were assigned to cohorts 4 (C4; previously treated) or 5b (C5b; treatment-naïve), independent of *MET* amplification status. Retrospectively, *METex14* positive and prescreen failed negative baseline FFPE tissue samples were tested using a hybrid capture DNA-based NGS assay (FoundationOne®). The *METex14* positive pts by DNA NGS were defined as having *MET* alterations that are predicted to lead to *MET exon 14* skipping.

Results: Of the 97 enrolled pts from the *METex14*-mutated cohorts C4 (n=69) and C5b (n=28) of the GEOMETRY mono-1 study, 73 pts had baseline tumor biopsy samples (C4, n=53; C5b, n=20) that met the requirements for the FoundationOne® NGS assay (minimum requirements: tissue volume ≥0.1 mm^3, DNA yield ≥ 22 ng, percent tumor nuclei ≥10). The FoundationOne® NGS assay identified *METex14* in 72 of 73 positive pts, with a concordance of 99% to the qualitative RT-PCR test used previously for testing. The variants detected included 41 unique canonical alterations that are predicted to lead to *METex14*. 1 pt with only a noncanonical *METex14* rearrangement was not included in the concordance analysis and reported stable disease. None of the RT-PCR negative patients were reported as positive by NGS.

Conclusions: Detection of *MET* exon 14 skipping events can be achieved by sequencing DNA or RT-PCR. A very high concordance was observed between DNA-based hybrid-capture NGS and RNA-based RT-PCR in the detection of *METex14* in FFPE tumor tissue from advanced NSCLC pts. NGS enables parallel detection of actionable alterations without sequential testing by single gene. Furthermore, this technique provides a comprehensive genomic profile to inform treatment plan and any potential mechanisms of resistance.

B12 FOXA2 promotes the growth of KRAS-mutant lung tumors but suppresses the growth of EGFR-mutant lung tumors in vivo. K. Tomoshige, W. Stuart, I. Fink-Baldauf, Y. Maeda. Cincinnati Children's Hospital, Cincinnati, OH.

Background: Using GEMM (genetically engineered mouse models), we showed that a lung-lineage transcription factor NKX2-1 promotes the growth of EGFR-mutant lung tumors but suppresses the growth of KRAS-mutant lung tumors in vivo (Maeda et al., JCI 2012), suggesting that such transcription factors expressed in the lung act as a context-dependent tumor promoter or suppressor. Here, we report the roles of a pioneer transcription factor FOXA2 expressed in lung epithelium in KRAS-mutant or EGFR-mutant lung tumors in vivo.

Methods: Using doxycycline-regulatable GEMM expressing mutant KRAS or mutant EGFR along with FOXA2 in lung epithelium (CCSP-rtTA; otet-KrasG12D; otet-Foxa2 or CCSP-rtTA; otet-EGFR.L858R; otet-Foxa2), we assessed whether FOXA2 influenced the growth of KRAS-mutant or EGFR mutant lung tumors in vivo. The number and size of lung tumors were analyzed by microCT. The histology of the lung tumors was further analyzed by H&E and immunohistochemistry.

Results: FOXA2 induced an increase in volume but not the number of KRAS-mutant lung tumors associated with lung adenocarcinoma while FOXA2 reduced the volume and number of EGFR-mutant lung tumors in vivo. Phosphohistone H3 was increased in KRAS-mutant lung tumors but decreased in EGFR-mutant lung tumors by FOXA2. Caspase-3 was not affected. These results indicate that FOXA2 differentially influences the initiation and progression of lung tumor growth depending on the type of driver oncogenes (mutant KRAS vs. mutant EGFR) in part through proliferation but not apoptosis.

Conclusion: Transcription factors NKX2-1 and FOXA2 function as yin and yang to affect the growth of KRAS-mutant or EGFR-mutant lung tumors.

B13 Selectively targeting lung cancer with a novel small molecule that induces lethality through dual inhibition of disulfide reductases. F. D. Johnson[1], S. Jansen[1], A. Liu[1], C. Brandstädter[2], D. Lu[1], A. Nagelberg[1], D. Farnsworth[1], T. Sihota[1], J. An[3], G. C. Forcina[4], A. Prudova[3], J. Luu[1], P. H.B. Sorensen[1], H. Varmus[5], R. Somwar[6], S. J. Dixon[4], S. J.M. Jones[1], K. Becker[2], G. B. Morin[1], W. W. Lockwood[1]. [1]University of British Columbia, Vancouver, BC, Canada, [2]Justus Liebig University Giessen, Giessen, Germany, [3]BC Cancer, Vancouver, BC, Canada, [4]Stanford University, Stanford, CA, [5]Weill Cornell Medicine, New York, NY, [6]Memorial Sloan Kettering Cancer Center, New York, NY.

Lung cancer (LC) is the leading cause of cancer-related deaths worldwide, mainly due to the lack of effective

therapies. Through a screen of 189,290 small molecules, the compound LC Screen 3 (LCS3) that inhibits the growth of LC cells but not normal cells was identified. LCS3 is structurally unique and its mechanism of action is unknown. Twenty-six lung adenocarcinoma cell lines were screened, and all but two were found to be sensitive to LCS3 (IC50<5μM). Transcriptome and proteome profiling by microarray and SILAC, respectively, suggest that LCS3 strongly induces redox imbalance. The top four predicted upstream transcriptional regulators of LCS3-induced RNA expression changes all have key functions in the response to oxidative stress (NRF2, MAFK, CEBPB, and BACH1). We confirmed LCS3 induces NRF2 activation through Western blot and flow cytometry analyses using a stably expressed antioxidant response element GFP reporter. In addition, flow cytometry with oxidative stress sensor H2DCFDA detected reactive oxygen species (ROS) induction by LCS3 only in sensitive cell lines. Notably, the most resistant LC cell line, NCI-H1648, has biallelic functional loss of KEAP1, which negatively regulates NRF2-mediated cytoprotective gene expression. We confirmed that NCI-H1648 has low basal ROS and high basal expression of genes that support redox balance. *KEAP1* silencing and antioxidants including N-acetylcysteine partially rescued LCS3-induced cytotoxicity, which further implicates oxidative stress in the mechanism of LCS3-induced cell death. To elucidate the molecular targets of LCS3, we applied thermal proteome profiling (TPP), which identifies thermally stabilized protein binders with proteome-wide coverage, and identified 47 proteins that are putative binders of LCS3. Of the 47 TPP hits, 8 are enzymes that function in redox homeostasis. Through in vitro enzymatic assays of the top TPP hits, we discovered that LCS3 inhibits glutathione disulfide reductase (GSR) and thioredoxin reductase 1 (TXNRD1) through reversible, uncompetitive inhibition at low micromolar IC50s. In silico molecular docking suggests LCS3 interacts with the GSR homodimer interface, and our structure-activity relationship studies have identified the putative functional moiety on LCS3 necessary for both enzymatic inhibition and cellular toxicity. We found that Luperox, a direct-acting hydroperoxide source of ROS, sensitizes nonresponsive cells to LCS3, thus implicating ROS as a requirement for LCS3-mediated toxicity. We are currently investigating why nonresponsive cells are less dependent on the glutathione and thioredoxin pathways and how oncogenic transformation, and the inherent oxidative stress that coincides, confers sensitivity to dual disulfide reductase inhibition. Through this work, we aim to use LCS3 as a tool compound to characterize a cancer dependency that can be exploited for the benefit of LC patients with advanced tumors, for whom treatment is urgently needed.

B15 COP1 E3 ligase modulates response to oncogenic MAPK pathway inhibition. M. K. Mayekar, L. Lin, T. G. Bivona. University of California San Francisco, San Francisco, CA.

Oncogenic activation of the RAS-MAPK pathway drives several cancers, including a majority of non-small cell lung adenocarcinomas (LAD). RAS-MAPK pathway is activated in lung adenocarcinomas via diverse genetic alterations in upstream receptor tyrosine kinases such as EGFR and ALK as well as in RAS, BRAF, MEK, and the RAS GTPase activating protein (GAP) and tumor suppressor, NF1. Therapeutically targeting components of the RAS-MAPK pathway can lead to initial tumor responses in many patients. However, very few patients show complete responses despite harboring the targeted RAS-MAPK pathway activating genetic lesion in the tumor. Responses and hence patient survival can be improved by better characterizing the molecular basis of response and resistance to therapies targeting the RAS-MAPK pathway in lung adenocarcinomas. To identify modulators of response to MAPK pathway inhibition in lung adenocarcinomas, we conducted genetic screens in BRAF-driven human lung adenocarcinoma cells. This identified the E3 ubiquitin ligase COP1/RFWD2 as a previously unknown genetic modifier in lung adenocarcinomas. We found that depletion of COP1 and members of its protein complex, as well as proteasomal subunits, confers resistance to RAS-MAPK pathway inhibition in patient-derived lung adenocarcinoma cells with oncogenic RAS-MAPK signaling. Intriguingly, oncogenic targets of COP1 include critical MAPK pathway effectors such as ETV1. Hence, we tested if depletion of COP1 protects those MAPK pathway effectors from the impact of RAS-MAPK pathway inhibitors. COP1 depletion had a substantial impact on the levels of these effectors in the presence of RAS-MAPK small-molecule inhibitors in lung adenocarcinoma cells. Furthermore, we found that co-depletion of these transcription factors resensitized COP1-depleted cells to MAPK pathway inhibition. Upon analyzing the transcriptomic and signaling changes, we found that low levels of COP1 facilitate survival of lung adenocarcinoma cells upon inhibition of the RAS-MAPK pathway by buffering the cells from the impact of the MAPK pathway inhibitor and thereby sustaining prosurvival pathways. Additionally, depletion of COP1 in in vitro derived models of resistance also resensitized them to MAPK pathway inhibition. This study has furthered our understanding of the molecular basis of tumor cell resilience during initial treatment as well as of secondary treatment resistance. We are examining if COP1 also modulates response to MAPK pathway inhibition in vivo and if levels of COP1 could be a biomarker for predicting

response to RAS-MAPK pathway inhibitor therapy in patients.

B16 The ROS1 Cancer Model Project: A unique patient-driven partnership to accelerate research. A. C. Moore[1], L. Goldman[2], T. Tomalia[2], R. C. Doebele[3], C. M. Lovly[4], R. Chiaverelli[5], T. Addario[6], A. Sable-Hunt[6], B. Addario[1], J. Freeman-Daily[2]. [1]GO$_2$ Foundation for Lung Cancer, San Carlos, CA, [2]The ROS1ders, Mountain View, CA, [3]University of Colorado Denver, Aurora, CO, [4]Vanderbilt University Medical Center and Vanderbilt-Ingram Cancer Center, Nashville, TN, [5]Champions Oncology Inc., Hackensack, NJ, [6]Addario Lung Cancer Medical Institute, San Carlos, CA.

Background: *ROS1* rearrangements (ROS1+) are found in a wide variety of cancer types but are relatively uncommon, occurring in 1-3% of lung, gastric ,and ovarian cancers, as well as melanoma, cholangiocarcinoma, glioblastoma, and other tumor types. ROS1 has been studied primarily in lung cancer, where there are now several FDA-approved drugs to treat advanced ROS1+ lung cancer. The rarity of ROS1 fusions makes studying them more challenging, as patients are too geographically dispersed to support a traditional clinical research study. To address this challenge, the ROS1ders joined forces with a leading lung cancer advocacy organization, an international research consortium, industry, and leading academic investigators to focus efforts on this rare molecular subset of cancer.

Method: The ROS1 Cancer Model Project currently consists of two studies supported by the Addario Lung Cancer Medical Institute's research infrastructure and remote study capabilities. Patients are empowered to contact the study team directly and do not have to be seen at a specific site to participate in the studies and donate samples for research. Due to the sparsity of research tools available to study ROS1+ cancer, the first study focuses on creation of patient-derived xenograft (PDX) models while the second study supports creation of cell lines. The ROS1ders and GO$_2$ Foundation for Lung Cancer have effectively utilized social media to connect with ROS1+ patients across the globe to educate them about the opportunity to participate in these ongoing research efforts. Both studies are currently open to ROS1+ patients located in North America.

Results: The ROS1 Cancer Model Project has successfully demonstrated the feasibility and power of patient-driven research and cross-sector collaboration to implement an innovative study motivated by patient need. Since its launch, the project has effectively mobilized the international ROS1+ patient population to create new cancer models for this rare molecular subset. To date, over 30 patients have been screened, with five patients referred to the PDX study and eight patients referred to the cell line study. Together, these studies have led to the successful development of new murine and cell-line research tools and have resulted in a doubling of the preclinical models now available for ROS1 research.

Conclusion: Through unique partnerships, the ROS1ders have accelerated the creation of new cancer models that will further researchers' understanding of this rare molecular subset. The success of this collaboration highlights the power of patients in driving research and has laid the foundation for similar efforts by other patient groups. This effort is part of the larger Global ROS1 Initiative, which is working to address the ongoing needs of the international ROS1+ patient community.

B18 Structural insight into sensitivity and resistance of RET mutants to selpercatinib (LOXO-292). T. Shen, S. S. Terzyan, X. Liu, B. H. Mooers, J. Wu. University of Oklahoma Health Sciences Center, Oklahoma City, OK.

Selpercatinib (LOXO-292) is a RET-selective protein tyrosine kinase inhibitor (TKI) designated as breakthrough therapy by the United States Food and Drug Administration. However, structural detail of its binding to RET was elusive. Protein tyrosine kinase targeted therapies often encounter resistance due to on-target mutations. Knowledge of TKI binding and resistant mutants is important for continuous TKI pipeline development and disease management. We have identified a panel of selpercatinib-resistant RET mutants in a preclinical model and determined the co-crystal structure of RET-selpercatinib complex to 2.06-Å resolution. Unlike vandetanib or nintedanib that insert into the gate, selpercatinib anchors one end in the front cleft and wrap around the gate wall to access the back cleft without penetrating the gate between the gatekeeper residue Val-804 and the gate wall residue Lys-758. Consequently, the gatekeeper mutants RET(V804L/M) had minimal effect on selpercatinib sensitivity. Nevertheless, among others, selpercatinib interacts with hinge and β2 residues, and its hydroxymethylpropoxy group protrudes out of solvent front. Consistently, selpercatinib-resistant mutations were found at the hinge, β2, and solvent-front residues. Our study details how selpercatinib uses an uncommon binding mode to occupy both clefts to limit the impact of gatekeeper mutants but is liable to resistance of non-gatekeeper mutations.

B19 New potential targets of antibody-drug conjugates for small-cell lung carcinoma. T. Yotsumoto, Y. Matsumoto, K. Zokumasu, T. Ando, K. Maemura, Y. Amano, K. Watanabe, H. Kage, K. Kakimi, J. Nakajima, D. Takai. University of Tokyo Graduate School of Medicine, Tokyo, Japan.

Small-cell lung cancer (SCLC) remains one of the high-grade malignancies, whereas non-small cell lung cancer benefits from molecular target drugs or immune checkpoint inhibitors as a result of investigating driver mutations and immune microenvironment. However, novel driver mutation was not identified through genome-wide sequence analyses, resulting in invariable therapeutic strategy for SCLC. Therefore, we have to shift from cytotoxic agent and molecular target drug in the care of SCLC. In recent years, different approaches to hematologic malignancies and solid tumors were established in clinical situation. Antibody-drug conjugates (ADCs) are the key technique. In this study, we aimed to search new therapeutic targets for ADCs toward a paradigm shift in treatment and research of SCLC. We sought to transmembrane proteins of SCLC as new targets for ADCs with a computational-biologic approach. We demonstrated 565 genes were overexpressed on 51 small-cell lung cancer cell lines compared to 30 normal lung tissue samples by investigating gene expression profile available in open source of Cancer Cell Line Encyclopedia and National Center for Biotechnology Information (NCBI) with Human Genome U133 Plus 2.0 Array (ThermoFisher Scientific). Among the 565 genes, 31 genes manifested increased value of compensated fluorescence signal on average by 3 or more. Of the 31 genes, by investigating RNA sequence data for normal tissue in NCBI, we identified 7 genes expressed in limited organs. We adopted these 7 selected genes as candidates for new targets of ADCs. We examined these new target genes by evaluating in vitro cytotoxicity of corresponding monoclonal antibodies followed by secondary ADCs comprising PNU-159682, a derivative of nemorubicin, using SCLC cell lines with and without overexpression of these genes. Cytotoxicity assay targeting a certain transmembrane protein, one of the candidate molecules, showed distinct effect of secondary ADC, inducing a large amount of cell death in a concentration-dependent manner while secondary ADC following murine IgG isotype control exhibited lack of cytotoxicity. Secondary ADC targeting the protein showed about fourfold greater potency than that using murine IgG isotype control as a primary antibody (EC50 3.3 nM versus 13.0 nM). Conversely, CRISPR-Cas9 mediated knockout of the gene showed explicit loss of the cytotoxic effect. The expression of the gene in normal organs was examined using human total RNA, which demonstrated lower expression of the gene in many organs than in brain. The distribution of the gene expression is preferable in the viewpoint of reducing side effects of the ADC, which cannot cross the blood-brain barrier. We successfully estimated new targets for ADCs by investigating membrane proteins and narrowing these proteins with a computational-biologic approach. Through in vitro cytotoxicity assays, the protein-mediated ADC exhibited specific killing of SCLC cell lines overexpressing the gene, suggesting the gene can be a potential target of ADCs.

B20 Oncogene-mediated ERK signaling suppresses neuroendocrine transcription factors and facilitates cellular transformation in small-cell lung cancer through chromatin remodeling. Y. Inoue, W. Lockwood. British Columbia Cancer Research Centre, Vancouver, BC, Canada.

Background: In contrast to non-small cell lung cancer (NSCLC), small-cell lung cancer (SCLC) rarely harbors gene alterations that activate signaling through the receptor tyrosine kinase/RAS/RAF/MEK/ERK pathway. In addition, EGFR protein expression is universally lost during histologic transformation from mutant *EGFR*-driven lung adenocarcinoma (LUAD) to SCLC that occurs in a subset of patients that develop acquired resistance to EGFR tyrosine kinase inhibitors (TKIs), despite the original *EGFR* mutations being maintained in the transformed tumor. Based on these observations, we hypothesized that signaling through mitogen-activated protein kinases (MAPKs) is detrimental to SCLC tumors and suppresses the neuroendocrine (NE) differentiation program that is a hallmark of this lung cancer subtype. To test this, we induced MAPK signaling through expression of two LUAD driver oncogenes, $KRAS^{G12V}$ and $EGFR^{L858R}$, and assessed the impact on the phenotype and signaling profiles of SCLC.

Methods: $KRAS^{G12V}$ or $EGFR^{L858R}$ was exogenously expressed in an inducible manner in three SCLC cell lines (H2107, H82, and H524). Effects were characterized through microscopy, growth assays, gene expression and chromatin profiling, and Western blots of master NE transcription factors including insulinoma-associated protein 1 (INSM1), POU class 3 homeobox 2 (BRN2), achaete-scute hologue 1 (ASCL1), and neurogenic differentiation factor 1 (NEUROD1).

Results: Induction of mutant KRAS or EGFR caused transition from suspension to adherent phenotype that was reversed by pharmacologic inhibition of both

ERK and AKT. Moreover, whereas both oncogenes downregulated NE transcription factors, effects were more prominent after $KRAS^{G12V}$ induction, reflecting the difference in degrees of phospho-ERK levels. Inhibition of ERK completely rescued the repression of NE factors by $KRAS^{G12V}$ induction, and partial effects were observed through inhibition of the downstream effectors MSK/RSK. Notably, $KRAS^{G12V}$-mediated suppression of NE factors was restored by inhibition of the histone modifiers p300/CBP or KDM5A in a cell line-specific manner. ATAC-seq analyses are currently underway to examine the changes of chromatin accessibility after $KRAS^{G12V}$ induction +/- inhibition of ERK, MSK/RSK, or p300/CBP.

Conclusions: In SCLC, activation of ERK and AKT by mutant *KRAS* or *EGFR* causes phenotypic transition to a NSCLC-like state, and ERK is the central hub for the regulation of NE factors. Histone modifications by hyperactivated ERK play an important role in this process and are mediated via tumor-specific mechanisms. These findings provide a biologic basis for why SCLC lacks alterations in the MAPK pathway and shed light on the underlying mechanisms of histologic transversion of SCLC to and from NSCLC, which may play a role in TKI resistance.

B22 Development of a novel serum marker for detecting small cell lung cancer by targeting a Cell Adhesion Molecule 1 (CADM1). T. Ito, T. Funaki, H. Iwanari, G. Tanaka, T. Nagase, T. Hamakubo, Y. Murakami. University of Tokyo, Tokyo, Japan.

Small-cell lung cancer (SCLC) accounts for about 15% of lung cancer. Although SCLC often responds favorably to combined-modality chemotherapy at the initial treatment, resistant tumors develop rapidly, which makes SCLC one of the representative cancers refractory to any therapeutic approaches. Moreover, molecular targeting therapy has not been developed for SCLC so far. Therefore, novel approaches to the diagnosis and treatment of SCLC on the basis of molecular understanding would be prerequisite to control this refractory cancer. One of the most critical issues of SCLC is its early detection in the initial screening and after chemotherapy. For this purpose, progastrin-releasing peptide (ProGRP) and neuron specific enolase (NSE) are widely used for serum markers for detection of SCLC, although combination of ProGRP and NSE can detect at most 60% of SCLC. We have previously demonstrated that CADM1, a member of the immunoglobulin superfamily cell adhesion molecules, is highly expressed in around 75% of SCLC. In addition, SCLC expresses a splicing variant, CADM1v8/9, which is observed specifically in normal testis. Here, we report that the extracellular fragment of CADM1v8/9 is digested by ADM17 and released into cell medium or human serum. Then, we generated specific monoclonal antibody against CADM1v8/9 using *Cadm1*-deficient mice and developed a serum diagnostic marker for SCLC. Preliminary study shows that CADM1v8/9 detects 47% of SCLC, which is independent of and partly overlaps with the cases detected by ProGRP. CADM1v8/9 can also detect a significant portion of patients with limited disease of SCLC. Furthermore, the amount of CADM1v8/9 fragments correlates well with the disease activity of SCLC before and after the chemotherapy. These findings indicate that detection of CADM1v8/9 in serum from patients is a novel and promising approach to detect and follow up SCLC patients. CADM1 would also provide a promising target for the treatment of SCLC.

B23 Unraveling the mechanisms of small-cell lung cancer brain metastasis. F. Qu[1], A. Pasca[1], C. Kong[2], M. Winslow[3], J. Sage[4]. [1]Department of Pediatrics, Stanford University School of Medicine, Stanford, CA, [2]Department of Pathology, Stanford University School of Medicine, Stanford, CA, [3]Department of Genetics, Department of Pathology, Stanford University School of Medicine, Stanford, CA, [4]Department of Pediatrics, Department of Genetics, Stanford University School of Medicine, Stanford, CA.

This abstract is being presented as a short talk in the scientific program. A full abstract is printed in the Proffered Abstracts section (PR08) of the Conference Proceedings.

B24 The role of cigarette smoke and miR520a in pulmonary Frizzled 9 expression. A. Smith, P. Do, M. Tennis. University of Colorado Anschutz Medical Campus, Aurora, CO.

Lung cancer is the deadliest cancer, and for this reason treatment is highly researched. Alternatively, chemoprevention can be used to combat lung cancer in individuals who are at high risk of diagnosis, such as cigarette smokers. Frizzled 9 (Fzd9) is required in vitro for chemopreventive effects of iloprost, a prostacyclin analogue, in the lung. In non-small cell lung cancer (NSCLC) cell lines, Fzd9 activates PPARγ, leading to inhibition of transformed growth. Cigarette smoke exposure decreases Fzd9 expression. The goal of this

study is to elucidate the relationship between cigarette smoke and miRNA regulation of Fzd9 expression. NSCLC cells exposed to cigarette smoke condensate (CSC) showed decreased Fzd9 3' UTR activity, suggesting CSC regulates Fzd9 expression through miRNA. miRNA database analysis suggested miR-95, miR-106b, and miR-520a as potential regulators of Fzd9. Immortalized human bronchial epithelial cells (HBEC) and an Fzd9-positive NSCLC cell line (A549) transfected with miR-520a oligonucleotide mimic showed decreased Fzd9 3'UTR luciferase activity. miR-520a expression increased in HBEC and NSCLC cells after CSC exposure. We have tested a miR-520a inhibitor to use for future rescue experiments in an Fzd9-negative cell line (H322). Transient overexpression of miR-520a in HBEC and A549 did not affect cell viability or proliferation, so we made a stable miR-520a expressing HBEC line that we will use for longer-duration cell assays. miR-520a may play an important role in the regulation of Fzd9 by cigarette smoke, and future experiments will characterize this relationship and potentially impact the application of iloprost chemoprevention.

B25 Mapping the SOX2 functional network in small-cell lung cancer. M. J. Vande Kamp[1], D. G. May[1], E. Thompson[1], H. Wollenzien[2], K. J. Roux[1], M. S. Kareta[1]. [1]Sanford Research, Sioux Falls, SD, [2]University of South Dakota, Vermillion, SD.

Small-cell lung cancer (SCLC) is a devastating and often recurring disease for which there has been little change in standard-of-care treatment over the last decade. Despite the advancement of cancer therapeutics, SCLC still has a five-year survival rate of less than 7%. By elucidating the genes and protein networks that drive SCLC tumor formation and growth, new avenues for treatment can be discovered. The transcription factor, SOX2, maintains stem cell pluripotency and is required for embryonic development. We have shown that SOX2 is a driver of SCLC. The SOX2 interactome has been studied in stem cells; however, in SCLC, the network of genes and proteins that SOX2 interacts with is still unknown. Here we present SOX2 chromatin targets as determined by chromatin immunoprecipitation (ChIP-seq) and CUT&RUN and compared binding to various epigenetic marks. The identification of SOX2 post-translational modifications suggests that they may impact its function in SCLC. Furthermore, the detection of SOX2 proximal proteins through BioID shows that SOX2 interacts with known regulators of lung cancer. As transcription factors are notoriously difficult to target therapeutically, our description of the SOX2 network presents novel targets for therapeutic development in SCLC.

B26 Relationship of *Sox2* and *Rb* in tumor initiation and maintenance in small-cell lung cancer. E. Voigt[1], H. Wollenzien[2], E. Thompson[1], J. Feiner[1], M. Vande Kamp[1], M. S. Kareta[1]. [1]Sanford Research, Sioux Falls, SD, [2]Sanford Research/University of South Dakota, Sioux Falls, SD.

Some cancer prognoses have been radically improved in recent years, but little headway has been made with others. One of these diseases, small-cell lung cancer (SCLC), has a five-year survival rate of less than 7% and a standard of care that has been essentially unchanged for forty years. One promising avenue to improve SCLC outcomes is to understand the cancer's underlying genetic alterations that drive its formation and growth. Functional inactivation of the *Rb* gene is seen in a number of cancers and is a genetic hallmark of SCLC. Normally *Rb* promotes differentiation by regulating lineage-specific transcription factors, including pluripotency factors such as *Sox2.* However, there is evidence that when certain tissues lose *Rb, Sox2* becomes upregulated and promotes oncogenesis. To understand this relationship in the pursuit to uncover new treatments for SCLC, we have studied the role of *Sox2* in *Rb* loss-initiated tumors by investigating both the tumor initiation in a SCLC genetically engineered mouse model, as well as tumor maintenance in SCLC cell lines and organoid culture.

B27 IHH acts as a tumor suppressor of lung adenocarcinoma by repressing reactive oxygen species. Sahba Kasiri[1], Baozhi Chen[1], Alexandra Wilson[1], Annika Reczek[1], Simbarashe Mazambani[2], Jashkaran Gadhvi[2], Evan Noel[1], Ummay Marriam[1], Barbara Mino[3], Wei Lu[3], Luc Girard[1], Luisa Solis[3], Katherine Luby-Phelps[1], Justin Bishop[1], Jung-whan Kim[2], James Kim[1]. [1]UT Southwestern Medical Center, Dallas, TX, [2]The University of Texas at Dallas, Dallas, TX, [3]The University of Texas MD Anderson Cancer Center, Houston, TX.

This abstract is being presented as a short talk in the scientific program. A full abstract is printed in the Proffered Abstracts section (PR09) of the Conference Proceedings.

B28 Intermittent hypoxia exacerbates tumor progression in a mouse model of lung cancer. S. H. Lee, H. S. Kang, I. K. Kim, C. D. Yeo, S. W. Kim, H. H. Kang, W. H. Ban. College of Medicine, The Catholic University of Korea, Seoul, Republic of Korea.

Background: Obstructive sleep apnea (OSA) is a very prevalent disorder characterized by chronic intermittent hypoxia (CIH), and some reports suggested that OSA is related to increased incidence of cancer as well as cancer progression. The purpose of this study was to evaluate whether obstructive sleep apnea (OSA)-related chronic intermittent hypoxia (CIH) influences lung cancer progression and to elucidate the associated mechanisms in a mouse model of lung cancer.

Methods: C57/BL6 mice in a CIH group were exposed to intermittent hypoxia for two weeks after tumor induction and compared with control mice (room air). Hypoxia inducible factor 1α (HIF-1α), vascular endothelial growth factor (VEGF), and metastasis-related matrix metalloproteinases (MMP) were measured. The expression levels of several hypoxia-related pathway proteins including HIF-1α, Wnt/ß-catenin, the nuclear factor erythroid 2-related factor 2 (Nrf2), and mammalian target of rapamycin-ERK were measured by Western blot.

Results: The number ($P < 0.01$) and volume ($P < 0.05$) of tumors were increased in the CIH group. The activity of MMP-2 was enhanced after CIH treatment. The level of VEGF was increased significantly in the CIH group ($p < 0.05$). ß-Catenin and Nrf2 were translocated to the nucleus and the levels of downstream effectors of Wnt/ß-catenin signaling increased after IH exposure.

Conclusions: CIH enhanced proliferative and migratory properties of tumors in a mouse model of lung cancer. ß-Catenin and Nrf2 appeared to be crucial mediators of tumor growth. These results suggest evidence for the causal link between OSA and lung cancer progression.

B30 The role of SMARCA4 as an EGFR-independent mechanism of resistance to osimertinib. F. J. de Miguel, B. Hu, W. L. Cai, N. Sun, M. C. Melnick, D. X. Nguyen, A. Z. Xiao, K. A. Politi. Yale University, New Haven, CT.

Targeted therapies have replaced conventional chemotherapy as first-line treatment for patients with non-small cell lung cancers harboring epidermal growth factor receptor (EGFR) alterations. Although tyrosine-kinase inhibitors (TKI) targeting these proteins lead to responses in ~70% of cases, tumors almost inevitably become resistant. Acquired resistance is commonly caused by secondary mutations in the target oncogene, activation of bypass signaling pathways, histologic transformation of the tumor, or unknown mechanisms (~20-40%). Epigenetic mechanisms are responsible for regulating genes involved in cell lineage specificity, and they are known to modulate tumorigenesis. In recent years, several epigenetic modifiers have also been implicated in processes related to drug resistance. We hypothesized that dysfunction of epigenetic processes plays a role in mediating resistance to TKIs. To examine this possibility, we generated three isogenic osimertinib-sensitive/resistant cell line pairs and mined whole-exome and RNA sequencing (RNA-seq) data. Distinct alterations and phenotypes were identified in the different models, highlighting the importance of the baseline biologic context for the type of osimertinib resistance mechanism that emerges. Using RNA-seq data, we searched for epigenetic regulators that might be mediating the differentially expressed genes in the resistant cells. This analysis revealed that the chromatin remodeling protein SMARCA4/BRG1 is required for maintenance of the resistant phenotype in one of the models as knockdown of BRG1 sensitized cells to osimertinib. Further analysis revealed that SMARCA4 is stabilized in TKI-resistant cells, thus leading to TKI resistance. Finally, immunohistochemistry (IHC) examination of a collection of TKI-resistant patient-derived xenografts (PDXs) revealed higher levels of SMARCA4 expression in TKI-resistant tumors without on-target EGFR-dependent resistant mechanisms. To further elucidate the role of SMARCA4, we are currently performing ATAC-seq experiments that will offer insights into chromatin accessibility mediated by the protein in the resistant cells. In addition, we are assessing the protein levels of SMARCA4 in clinical specimens obtained before treatment and at the time of resistance by IHC. As new and better targeted therapies are developed, complex resistance mechanisms that involve epigenetic changes in tumors are likely to be increasingly observed. Our studies offer insights into the mechanisms that underlie such resistance that could lead to new therapeutic possibilities for these tumors.

B31 Development of multicell type organoid cultures for preclinical studies of immunotherapeutics for lung cancer. J. Flaming, L. Girard, R. Brekken, J. Minna. UT Southwestern Medical Center, Dallas, TX.

Introduction/Purpose of Study: Macrophages are key regulators of the immune landscape within the tumor microenvironment (TME). The plasticity of macrophage phenotypes in the TME has previously been correlated with prognosis within non-small cell

lung cancer (NSCLC). Depending on their phenotype, macrophages in the TME can secrete protumor cytokine and chemokines, ultimately suppressing the function of other immune cells in the TME. The purpose of our study was to explore the ability of individual NSCLC preclinical models to alter macrophage phenotype in organoid cultures and to relate effects on macrophages to the molecular characteristics of different NSCLCs. We hypothesize that immune suppression occurs through tumor-secreted signaling molecules, and if blocked, macrophage suppression can be alleviated, resulting in a better antitumor immune response.

Experimental Procedures: We developed an in vitro organoid coculture system (NSCLC tumor cells, human cancer-associated fibroblasts, CAFs, and mouse macrophages) to interrogate cancer cell features causing heterogeneity of macrophage phenotypes across a panel of NSCLCs. We measured (with 4-7 replicates for each NSCLC): mRNA expression in mouse macrophages with a panel of qPCR probes for important macrophage-related genes (Arg, NOS2, IL1beta, IL-6, CHIL-3, SOCS3), and in selected cases whole-genome RNAseq; and protein expression using cytokine arrays measuring expression of 40 inflammatory cytokines. Positive controls were stimulation with LPS and IL-4.

Summary of New Data: Using our platform, we characterized 70 NSCLC patient-derived lines by their ability to alter mouse macrophage phenotype. We found: 1. the macrophage phenotypes induced by any one NSCLC were highly reproducible; 2. three major clusters of cancer polarized macrophage phenotypes: high Arg (immune suppressive), high IL-1beta (inflammatory) or high SOCS3 (cGAS-STING pathway) expression; and 3. the major oncogenotypes (KRAS, TP53, STK11, EGFR, BRAF) have no correlation to the induced macrophage phenotype. We selected 7 NSCLC "exemplar" lines representing each of these 3 clusters for RNA sequencing (mouse genes) and cytokine array protein (human) profiling. Across all clusters we found: 1. suppression of macrophage endocytosis pathways and activation of scavenger receptor A (SRA) signaling (M2 immune suppressive phenotype); and 2. increased expression of human IL6, IL8, and MCP1 proteins, which have been implicated in suppressing innate immune tumor sensing. Analyses of differences between the 3 clusters is ongoing.

Conclusions: Patient-derived NSCLC preclinical models have reproducible effects on altering macrophage phenotypes in organoid cultures. Three major classes of NSCLC initiated macrophage alteration, which are not linked to oncogenotype. Cytokines secreted by the NSCLCs appear responsible for these macrophage changes, and this system provides an experimental mechanism to systematically test each as potential therapeutic targets.

B32 Drug sensitivity and allele specificity of first-line osimertinib resistance *EGFR* mutations. J. H. Starrett[1], A. Guernet[2], M. E. Cuomo[2], K. Poels[3], I. K. van Alderwerelt van Rosenburgh[1], A. Nagelberg[4], D. Farnsworth[4], K. Price[5], H. Khan[6], K. D. Ashtekar[1], M. Gaefele[1], D. Ayeni[1], T. F. Stewart[1], A. Kuhlmann[1], S. M. Kaech[7], A. M. Unni[8], R. Homer[1], W. W. Lockwood[4], F. Michor[3], S. B. Goldberg[1], M. A. Lemmon[1], P. Smith[2], D. Cross[2], K. Politi[1]. [1]Yale School of Medicine, New Haven, CT, [2]AstraZeneca, Cambridge, United Kingdom, [3]Harvard T.H. Chan School of Public Health, Dana-Farber Cancer Institute, Boston, MA, [4]British Columbia Cancer, University of British Columbia, Vancouver, BC, Canada, [5]Guardant Health, Redwood City, CA, [6]Brown University, Lifespan Cancer Institute, Providence, RI, [7]The Salk Institute, La Jolla, CA, [8]Weill Cornell Medicine, New York, NY.

Osimertinib, a mutant-specific third-generation EGFR TKI, is emerging as the preferred first-line therapy for *EGFR* mutant lung cancer. Despite initial responses in patients, however, resistance inevitably develops over time. In order to investigate mechanisms of resistance to first-line osimertinib, we modeled acquired resistance to this drug in transgenic mouse models of $EGFR^{L858R}$-induced lung adenocarcinoma and found that it is mediated largely through secondary mutations in *EGFR* – either C797S or L718V/Q. Analysis of circulating free DNA data from patients with *EGFR* mutant lung cancer revealed that L718Q/V mutations almost always arise in the context of an L858R driver mutation, and may occur at least as frequently as C797S in T790M-negative tumors. Therapeutic testing in mice revealed that both erlotinib and afatinib caused regression of osimertinib-resistant C797S-containing tumors, whereas only afatinib was effective in L718Q mutant tumors. Combination first-line osimertinib plus erlotinib treatment prevented the emergence of secondary mutations in *EGFR*. Finally, we report a patient with a tumor harboring both the L718V and L718Q mutations at resistance to first-line osimertinib who benefited from afatinib treatment. Our data identify specific secondary *EGFR* mutations as a major mechanism of acquired resistance to first-line osimertinib treatment and highlight potential strategies to overcome or prevent osimertinib resistance in vivo. Furthermore, these findings emphasize how knowledge of the specific characteristics of resistance mutations is important for determining potential subsequent treatment approaches.

B33 Short-term exposure to REV-5901 decreases the viability of chemotherapy-resistant adherent lung cancer cells and floating tumorspheres. J. S. Yakisich[1], R. Venkatadri[1], B. Brinceanu[2], C. Woodard[1], V. Kaushik[1], N. Azad[1], A. K. V. Iyer[1]. [1]Hampton University, Hampton, VA, [2]Governor's School for Science & Technology, Hampton, VA.

Toxicity to normal cells as well as the presence of highly resistant cancer cells, such as cancer stem-like cells (CS-LCs), are key factors that limit the efficacy of chemotherapy. In tumors, CS-LCs are often associated with chemoresistance and tumor relapse. In this study we used two models of highly resistant lung cancer cells: 1) Adherent cells (anchorage-dependent) growing under prolonged periods of serum starvation (PPSS) and 2) cells growing as floating (anchorage-independent) tumorspheres (FTs) to evaluate the effect of REV 5901. Cell viability was determined by the MTT or the CCK assay for adherent cells and FTs, respectively. Protein levels were determined by Western blots. Compared to cells growing under routine culture conditions (RCCs), cells growing under PPPS or as FTs were highly sensitive to REV. REV was able to selectively and irreversibly decrease the viability of cells growing under PPSS or as FTs within 24 h. Recovery experiments exposing cells to REV for 24 h followed by incubation in drug-free media for 48 h demonstrated that while PPSS as well as FTs cells were unable to recover, the noncancerous cell line Beas-2B growing under RCCs was not only less sensitive to REV but was also able to recover significantly. At the molecular level, REV induced significant changes in the expression of key proteins of the Wnt signaling pathway. Our data demonstrate that short treatment with REV can eliminate highly resistant cancer cells and that the Wnt signaling pathway may play a central role.

B34 Combination therapy with Wnt pathway modulators to override chemoresistance in human lung cancer cells. J. S. Yakisich, V. Kaushik, A. R. Guishard, D. Afolabi, N. Azad, A. K. V. Iyer. Hampton University, Hampton, VA.

Background: The serum levels of DDK1, a negative regulator of the Wnt signaling pathway, have been reported to be elevated in cancer patients. DKK1 expression and association to chemoresistance has not been extensively investigated in cancer stem-like cells. In this study, by using cancer cell lines growing under anchorage-dependent conditions (Adherent cells; chemosensitive phenotype) as well as cells growing under anchorage-independent conditions (Floating Spheroids [FSs]; chemoresistant phenotype), we evaluated a) the expression of DKK1 and the downstream effector of the Wnt signaling pathway β-catenin and b) the effect of iCRT-14 (a β-catenin inhibitor) and WAY-262611 (a DKK1 inhibitor) on the viability of cancer cells.

Methods: FSs were grown in ultra-low attachment plates for 7 days. Cell viability were determined by the MTT or the CCK assay for adherent cells and FTs, respectively. Protein levels were determined by Western blots.

Results: A549 and H460 adherent cells were sensitive to both iCRT-14 and WAY-262611. FSs generated from these cell lines were resistant to WAY-262611 but still sensitive to iCRT-14. FSs prepared from H460 cells were more sensitive to iCRT compared to FSs prepared from A549 cells. Western blot analysis from protein lysates prepared from H460 cells showed that iCRT-14 decreased the expression of β-catenin.

Conclusions and Future Directions: Our data demonstrate that a DKK-1 inhibitor in combination with a β-catenin inhibitor has the potential to eliminate lung cancer cells displaying varying degrees of chemoresistance. We are currently characterizing the mechanism by which this combination modulates the Wnt signaling pathway.

B35 Circulating tumor-associated cells in lung cancers are resistance-educated per previous chemotherapy treatments. Dadasaheb B. Akolkar[1], Sewanti Limaye[2], Darshana Patil[1], Sanket Patil[1], Vishakha Mhase[1], Sachin Apurwa[1], Sushant Pawar[1], Vipul Todarwal[1], Vineet Datta[1], Cynthe Sims[1], Ajay Srinivasan[1], Rajan Datar[1]. [1]Datar Cancer Genetics Limited, Nasik, Maharashtra, India, [2]Kokilaben Dhirubhai Ambani Hospital, Mumbai, India.

Resistance to chemotherapy agents is frequently encountered in non-small cell lung cancers (NSCLC) and is largely undetected until symptomatic or radiologic detection of disease progression. Real-time monitoring of chemoresistance in NSCLC is an unmet clinical need. We describe a novel approach for real-time chemoresistance profiling (CRP) in NSCLC using peripheral blood circulating tumor-associated cells (CTACs), which are apoptosis-resistant cells of tumorigenic origin (EpCAM+, pan-CK+, CD45±). Peripheral blood was collected from 145 patients with confirmed NSCLC including 102 therapy-naïve cases and 43 pretreated cases. Peripheral blood mononuclear cells (PBMCs) were harvested by centrifugation. C-TACs were enriched using an epigenetically activated

medium that eliminates normal (nontumorigenic) cells and confers survival privilege on apoptosis-resistant tumorigenic cells (C-TACs). Surviving C-TACs were confirmed by immunostaining (EpCAM, pan-CK, CD45, TTF-1, Napsin-A). Harvested C-TACs were treated in vitro with a panel of conventional cytotoxic agents and the fraction of surviving cells estimated to determine resistance profiles. Among the therapy-naïve NSCLC, innate chemoresistance towards any agent was observed in 51.7% of cases, which included resistance towards platinum agents in 37.8% of cases, microtubule targeting agents in 54.5% of cases, antimetabolites in 57.1% of cases, and topoisomerase inhibitor in 57.3% of cases. Among the pretreated NSCLC cases, resistance towards any agent was observed in 88.1% of cases, which included resistance towards platinum agents in 84.9% of cases, microtubule targeting agents in 85.1% of cases, antimetabolites in 96.7% of cases and topoisomerase inhibitor in 100% of cases, respectively. In vitro chemoresistance profiling of C-TACs is a viable approach for real-time monitoring of innate and acquired chemoresistance. Higher chemoresistance in the pretreated population, as compared to the therapy-naïve population, indicates that C-TACs are resistance-educated by prior treatments.

B36 Effects of trifluoperazine and its analog on A549 human lung cancer cells. J. Jeong, J. Park, N. Park, G. Kang, S. S. Kang. Gyeongsang National University, Jinju, Korea.

Although there have been great advances in technology, molecular diagnosis, and therapeutics, lung cancer is still the leading cause of cancer-related mortality all over the world. Recently, some antipsychotic drugs have been shown to possess anticancer activity. Thus, the present study was designed to evaluate the anticancer effects of trifluoperazine (TFP), a commonly used antipsychotic drug, and its synthetic analogs on human lung cancer cell lines. To this end, effects of TFP and its selected analog on A549 cells were investigated in in vitro as well as in vivo experiments. Synthetic TFP analogs were evaluated by the proliferation of A549 cells following drug treatment and compared to TFP. 3dc, a selected TFP analog, significantly inhibited the proliferation of A549 cells. Further experiment showed that TFP and 3dc had activities to inhibit the anchorage dependent/independent colony formation, and migration of A549 cells. Western blot analysis revealed that 3dc affected the gene expression levels related to apoptosis and cell cycle. Flow cytometric analysis showed that 3dc induced sub-G1 and G1 population and reduced cell population in S and G2/M phase. Additionally, Annexin V/PI staining showed that 3dc increased apoptotic cell population. Moreover, 3dc increased DNA fragmentation. 3dc showed stronger anticancer effects in all the experiments than TFP. In addition, in in vivo experimental models, 3dc also showed a powerful anticancer effect in orthotropic lung cancer development than TFP. Thus, the present study demonstrates that a synthetic TFP analog has anti-lung cancer activity and provides a potential therapeutic candidate for lung cancer.

B38 Serum albumin as an independent prognosis factor in patients with non-small cell lung cancer by affecting the distribution of CD8+ T cells. Lingyu Li, Haishuang Sun, Xiao Chen, Dongsheng Xu, Jiuwei Cui. The First Hospital of Jilin University, Changchun, Jilin, China.

Background: Serum albumin (ALB) as the most common biomarker for nutritional status is often closely associated with the prognosis of patients with non-small cell lung carcinoma (NSCLC). Whether the cause is related to the effects on host immune status, especially for distribution of host immune cells, remains currently unknown.

Patients and Methods: Clinical data, peripheral blood (PBL), and tumor tissues were obtained from enrolled patients with primary NSCLC in the First Hospital of Jilin University. We performed flow cytometry to analyze the PBL immunocytes and quantitative immunofluorescence to detect the tumor-infiltrating CD8+ T cells. TCR repertoire analysis was examined by high-throughput sequencing of TCR β-chain. All the clinical outcomes, correlations between ALB, and immune indexes were analyzed by SPSS 17.0.

Results: In the total of 211 enrolled NSCLC patients, ALB became an independent prognostic factor through multivariate Cox regression analysis (P=0.037). The median OS and PFS in patients with low ALB (N=155) vs. high ALB (N=56) were 28.2 vs. 42.2 months (P=0.0142), and 14.6 vs. 25 months (P=0.0149), respectively. Among patients with non-metastasis NSCLC (stage I-III), there was a higher incidence rate of distant metastasis in low ALB group than that in high ALB group (41.3% and 22.2%; P=0.043), in addition to a strong association with higher risk of death (P<0.01) and disease progression (P=0.037). We further found that high ALB was closely correlated with higher PBL cholesterol (r=0.4189, P<0.0001), triglyceride (r=0.2302, P=0.0008) and HDL (r=0.2849, P< 0.0001), resulting in more CD8+ cytotoxic T cells in PBL (P=0.007) and around the tumor (P=0.047)

but not infiltrated in tumor. Furthermore, high ALB also associated with more diversity of TCR repertoire (P=0.023).

Conclusions: High ALB improved the survival and reduced risk of distant metastasis in NSCLC patients by affecting the distribution of CD8+ T cells and diversity of TCR repertoire.

B39 Cancer and palliative care in rural India (West Bengal): Experience of an NGO. Aditya Manna. Narikeldaha Prayas, East Medinpur, West Bengal, India.

Introduction: As in any developing countries, the state of West Bengal in India has a huge burden of cancer patients in advanced stage coming from rural area where awareness regarding the usefulness of palliative care in rather poor.

Objective: Our goal is to give a pain-free good quality of life in these advanced-stage cancer patients. The objective of this study is to identify the main difficulties in achieving the above goal in a rural village setting in India.

Method: Advanced cancer patients in need of palliative care in various villages in rural India were selected for this study. Their symptoms and management in those rural surroundings were evaluated by an NGO (under the guidance of a senior palliative care specialist) working in that area. An attempt was made to identify the main obstacles in getting proper palliative care in a rural setting.

Results: Pain and fatigue are the main symptoms affecting these patients. In most patients pain and other symptoms' control were grossly inadequate due to lack of properly trained manpower in rural India. However, regular home care visits by a group of social workers were of immense help in the last few months of life. The NGO team was well guided by a palliative care specialist.

Conclusion: There is a wide gap of trained manpower in this field in rural areas of India. Dedicated groups from the rural area itself need encouragement and proper training, so that difficult symptoms can be managed locally along with necessary social and psychological support of these patients.

B40 IGF-binding protein-mediated sensitization of EGFR-mutant NSCLC cells to osimertinib by cancer-associated fibroblast. L. L. Remsing Rix[1], N. J. Sumi[1], A. T. Bryant[1], B. Desai[1], X. Li[1], E. A. Welsh[1], B. Fang[1], B. M. Kuenzi[1], S. J. Antonia[1], C. M. Lovly[2], J. M. Koomen[1], A. Marusyk[1], E. B. Haura[1], U. Rix[1]. [1]Moffitt Cancer Center, Tampa, FL, [2]Vanderbilt-Ingram Cancer Center, Nashville, TN.

Background: Cancer-associated fibroblasts (CAFs) are known to be able to support tumor growth, metastasis, and drug resistance. However, in resistant EGFR mutant lung cancer cells we also observed noncanonical CAF-driven sensitization to specific targeted drug treatment. Elucidation of the underlying mechanisms may identify novel biomarker or drug combination approaches.

Methods: Viability of EGFR-mutant, gefitinib-resistant PC9GR cells in coculture or in the presence of CAF conditioned medium (CM) was monitored by live-cell imaging using the IncuCyte system or via CellTiterGlow (CTG, Promega), respectively. Clonogenic assays were analyzed by crystal violet staining. Gene expression differences of CAFs vs. normal activated fibroblasts (NAFs) were determined by microarrays. Secreted proteins in the CM were identified by mass spectrometry-based proteomics. Signaling changes were monitored by RTK array, phosphoproteomics, and Western blot. Loss- and gain-of-function experiments were performed using siRNA, small-molecule inhibitors, or addition of recombinant human (rh) proteins. Drug combinations were evaluated by CTG, crystal violet, and mouse xenografts.

Results: Gene expression and secretome analysis of CAFs vs NAFs identified differential expression of secretory molecules, in particular IGF1 and 2 and IGF-binding proteins (IGFBPs), which regulate IGF1R signaling, a pathway linked to EGFR inhibitor resistance. RTK arrays and phosphoproteomics showed enhanced inhibition of IGF1R and ERK phosphorylation by osimertinib in the presence of CAF CM. Consistently, combination of IGF1R and EGFR inhibitors closely mimicked the effect of EGFR inhibition in the presence of CAF CM. CM from CAFs where IGFBPs were silenced by siRNA or treatment with IGF1 or 2 partially rescued cells from osimertinib, while rhIGFBPs conversely mimicked CM sensitizing effects. CAF CM vs. NAF CM further reduced AKT and ERK phosphorylation upon EGFR inhibition. The combination effect of EGFR and IGF1R inhibition has been shown in several cell lines, in vivo, as well as with several different drug combinations.

Conclusion: We found CAF-mediated drug sensitization in EGFR-mutant lung cancer, which involves the IGF1R signaling axis. IGFBPs secreted from CAFs attenuate compensatory signaling, leading to improved EGFR inhibitor efficacy. This result highlights tumor-suppressive effects of CAFs competing with their tumor-promoting effects and adds to the growing evidence that eliminating CAFs in an undifferentiated way may be detrimental to cancer therapy. Rather, we show that mechanistic understanding of these suppressive pathways can lead to improved drug combinations that mimic these effects and may delay the onset of resistance.

B41 Translating lung cancer research into primary care provider training: An innovative online course. C. T. Worth, S. Sthapit-Gaines, K. F. Coombs, M. Jones, C. L. Sorrell. LuCa National Training Network, Louisville, KY.

Background: Lung cancer (LC) is the leading cause of cancer death in the U.S., and primary care provider (PCP) education is essential for reducing the country's overall LC burden. Despite recent LC treatment advances, several studies show that LC patients receive treatments at lower rates than other cancer patients, regardless of stage of diagnosis. Despite decades of research into tobacco use treatment, many PCPs still lack the expertise to assist patients in their quit attempts. PCP understanding and adoption of the U.S. Preventive Services Task Force (USPSTF) recommendation for LC screening with low-dose CT (LDCT) remain low, making PCP education crucial for impacting the leading cause of cancer death.

Methods: The LuCa National Training Network provides training, materials, and technical assistance for professionals who educate PCPs and other health care professionals on LC. In developing an online training course on lung cancer care for PCPs, LuCa moved beyond the traditional, using innovative technology to engage PCPs and influence practice behaviors. The free course, Lung Cancer and the Primary Care Provider, delivers comprehensive, interactive education on best practices related to lung cancer prevention, early detection, treatment, and survivorship. The course includes a library of practice tools available for download. This presentation will provide an overview of the online course and its translation of prevention research and treatment advances into practical applications for PCPs. Post-test results and provider feedback on the course will be shared.

Results: Lung Cancer and the Primary Care Provider launched in August 2019 and is the first comprehensive online course on LC care for PCPs in the U.S. The course already has participation from over 35 states and other countries. Up-to-date participation and evaluation trends, demographic comparisons, knowledge changes from pre- and post- tests, lessons learned, and self-reported practice behavior changes will be shared. This presentation will review the practice areas on which the course had the strongest influence, based on PCP self-reported data.

Conclusion: Adoption of new clinical practices takes time, but it can be accelerated by high-quality training that clearly outlines recent research, provides clear best practices for PCPs, and offers downloadable, evidence-based practice tools. PCPs play a pivotal role in influencing the entire lung cancer care continuum and determining the care pathways for their patients. They are an ideal audience for pertinent lung cancer-focused educational interventions. Creative, on-demand training has tremendous potential to improve patient care and achieve broad reach to this critical clinical audience. Provider education must be engaging, evidence-based, and cutting-edge in order to capture focus with competing demands and affect knowledge and confidence on complex lung cancer topics.

AUTHOR INDEX

D

E

F

AUTHOR INDEX

J

K

L

AUTHOR INDEX

M

N

O

P

Q

R

AUTHOR INDEX

S

T

U

V

W

AUTHOR INDEX

X

Y

Z

SUBJECT INDEX

H

I

K

L

M

N

DISCLOSURES OF FINANCIAL RELATIONSHIPS

In compliance with the standards set by the Accreditation Council for Continuing Medical Education (ACCME), it is the policy of the American Association for Cancer Research (AACR) that the information presented at CME activities will be unbiased and based on scientific evidence. To help participants make judgments about the presence of bias, the AACR has provided information that planning committee members, speakers, and abstract presenters have disclosed about financial relationships they have with commercial entities that produce or market products or services related to the content of this CME activity.

Relationships are abbreviated as follows: E, Employee of listed company, G, Grant/research support recipient, A, Advisor or review panel member, C, Consultant, S, Stock Shareholder, SB, Speakers' Bureau, H, Honoraria, O, Other.

Last Name	First Name	Company	Relationships	Type	Role
Ambrogio	Chiara	Dana-Farber Cancer Institute	No Relationships		Speaker
Anagnostou	Valsamo	Sidney Kimmel Cancer Center at Johns Hopkins	Bristol-Myers Squibb	G	Speaker
Arthur	Ronald	American Association for Cancer Research	No Relationships		Staff
Baran	Amy	American Association for Cancer Research	No Relationships		Staff
Barton	Colin	ALK Positive	No Relationships		Speaker
Beane-Ebel	Jennifer	Boston Univ. School of Medicine	Janssen, Veracyte	G,O	Speaker
Bivona	Trever	UCSF	Revolution Medicines, Novartis, AstraZeneca, Array Biopharma, Jazz	A,C,G	Program Committee, Speaker
Bruno	Tullia	Univ. of Pittsburgh School of Medicine	No Relationships		Speaker
Cooper	Jonathan	Genentech	Genentech	E	Speaker
Dayalan	Antony	H. Lee Moffitt Cancer Center & Research Institute	No Relationships		Speaker
DeNicola	Gina	H. Lee Moffitt Cancer Center & Research Institute	No Relationships		Speaker
Doebele	Robert	Univ. of Colorado Denver	Rain Therapeutics, Genentech/Roche, Takeda/Millenium, Bayer, AstraZeneca	A,C, H,S	Speaker
Farago	Anna	Massachusetts General Hospital	AstraZeneca, AbbVie, Boehringer Ingelheim, Bayer, Bristol-Myers Squibb, Roche, Genentech, Merck, Pharmamar, Amgen, Loxo	O	Speaker
Feldman	Jill	SBC Global, Inc.	AstraZeneca	C	Speaker
Feldser	David	Univ. of Pennsylvania	No Relationships		Speaker
Freeman-Daily	Janet	Bonnie J. Addario Lung Cancer Foundation	TP Therapeutics, Neogenomics	O,H	Speaker

Last Name	First Name	Company	Relationships	Type	Role
Garon	Edward	University of California (UCLA)	Merck, Iovance, Mirati, Dynavax, Neon, Novartis, EMD Serono, Dracen, AstraZeneca, BMS, Eli Lilly, Genentech	G	Speaker
Gibbons	Don	UT MD Anderson Cancer Center	Janssen R&D, Takeda, Ribon Therapeutics, AstraZeneca, Alethia	A,C,G	Speaker
Gillette	Michael	Broad Institute of MIT and Harvard	No Relationships		Speaker
Graeber	Thomas	University of California (UCLA)	Amgen, Trethera, ImmunoActiva	C,G, H,S	Speaker
Haura	Eric	H. Lee Moffitt Cancer Center & Research Institute	No Relationships		Speaker
Heymach	John	UT MD Anderson Cancer Center	AstraZeneca, Boehringer Ingelheim, Exelixis, Genentech, GSK, Guardant Health, Hengrui, Lilly, Novartis, Spectrum, EMD Serono, Synta, AstraZeneca, Bayer, GlaxoSmithKline, Spectrum, Takeda, Spectrum, Biotree	A,G,O	Program Committee, Speaker
Joshi	Nikhil	Yale University	No Relationships		Speaker
Kim	Jung-Whan	UT Dallas	No Relationships		Speaker
Lampe	Paul	Fred Hutchinson Cancer Research Ctr.	No Relationships		Speaker
Li	Rui	UCLA David Geffen School of Medicine	No Relationships		Speaker
Lockwood	William	BC Cancer Agency	No Relationships		Speaker
Lovly	Christine	Vanderbilt Univ. School of Medicine	Takeda, Foundation Medicine, Achilles, Roche, Blueprints Medicine, Cepheid	C,H	Program Committee, Speaker
Marron	Thomas	Mt. Sinai Medical Ctr. Tisch Cancer Inst.	BMS	G	Speaker
Massion	Pierre	Vanderbilt-Ingram Cancer Ctr.	No Relationships		Speaker
McCoach	Caroline	UCSF School of Medicine	Novartis, Takeda, Genentech, Revolution Medicines	A,G,H	Speaker
McFadden	David	UT Southwestern Medical Center	No Relationships		Speaker
Moorthi	Sitapriya	Fred Hutchinson Cancer Research Center	Amgen	S	Speaker
Nguyen	Don	Yale University, School of Medicine	AstraZeneca, Leidos	G	Speaker
O'Donnell	Kathryn	UT Southwestern Medical Center	No Relationships		Speaker
Okimoto	Ross	UCSF	No Relationships		Speaker

DISCLOSURES OF FINANCIAL RELATIONSHIPS

Last Name	First Name	Company	Relationships	Type	Role
Oliver	Trudy	University of Utah Huntsman Cancer Institute	No Relationships		Speaker
Ou	Sai-Hong	UC Irvine Chao Family Comp. Cancer Ctr.	TPTX, Pfizer, Roche/ Genentech, AstraZeneca, Takeda/ARIAD	A,C,H, S,SB	Speaker
Paik	Paul	Memorial Sloan Kettering Cancer Center	Celgene, Takeda, Boehringer Ingelheim, AstraZeneca, Calithera	A,H	Speaker
Pao	William	Roche	Roche	E	Speaker
Piotrowska	Zofia	Massachusetts General Hospital	AstraZeneca, Eli Lilly, InCyte, Genetech, ImmunoGen, Medtronic, Takeda, Novartis, Spectrum	A,G	Speaker
Poirier	John	Perlmutter Cancer Center at NYU Langone Health	Intellectual Property	O	Speaker
Politi	Katerina	Yale Cancer Center	Molecular MD, AZ, Koltan, Symphogen, Roche, Dynamo Therapeutics, Maverick Therapeutics	C,G,O	Program Committee, Speaker
Prabhu	Antony	H. Lee Moffitt Cancer Center	No Relationships		Speaker
Qu	Fangfei	Stanford University School of Medicine	No Relationships		Speaker
Robichaux	Jacqulyne	UT MD Anderson Cancer Center	Spectrum	O	Speaker
Salehi-Rad	Ramin	University of California (UCLA)	No Relationships		Speaker
Schalper	Kurt	Yale University	Clinica Alemana Santiago, Celgene, Moderna Therapeutics, Shattuck Labs, Pierre-Fabre, Astrazeneca, Dyanamo Therapeutics, EMD Serono, Takeda, Torque Therapeutics, Agenus, Genoptix/Navigate (Novartis), Vasculox/ Tioma, Tesaro, Onkaido Therapeutics, Takeda Pharmaceuticals, Surface Oncology, Pierre-Fabre Research Institute, Merck, Bristol-Myers Squibb, AstraZeneca, Eli Lilly, Merck, Bristol-Myers Squibb, Fluidigm	C,G, SB	Speaker
Shackelford	David	UCLA David Geffen School of Medicine	No Relationships		Speaker
Shaw	Reuben	Salk Institute	No Relationships		Speaker
Skoulidis	Ferdinandos	UT MD Anderson Cancer Center	No Relationships		Speaker
Sommers	Connie	National Cancer Inst.	No Relationships		Speaker

Last Name	First Name	Company	Relationships	Type	Role
Soucek	Laura	Vall d'Hebron Institute of Oncology (VHIO)	Peptomyc S.L.	A,S,O	Speaker
Wong	Kwok-Kin	New York University Langone Medical Center	Janssen, AZ, G1 Therapeutics, Genocea, Novartis, Pfizer, Takeda, Mirati, Merus, BMS	A,C, G,S	Speaker
Wood	Kris	Duke University	UCB/Element Genomics, Celldom, Tavros Therapeutics	A,C, G,S	Speaker
Wrangle	John	Medical University of South Carolina	No Relationships		Speaker

NOTES

NOTES

NOTES

NOTES

AACR American Association for Cancer Research

ANNUAL MEETING 2020 • SAN DIEGO

APRIL 24-29

TURNING SCIENCE INTO LIFESAVING CARE

Join us in San Diego for the latest innovative and inspiring cancer research from around the world...**the AACR ANNUAL MEETING 2020!**

Late-Breaking Abstracts and Clinical Trials Abstract Submission Deadline: Thursday, January 30, 2020

Advance Registration Deadline: Friday, February 21, 2020

Become a Member!
Join the AACR and receive a discount on registration.

ABIM CME MOC ACCREDITED

Continuing Medical Education Activity - *AMA PRA Category 1 Credits*™ available

The AACR Annual Meeting highlights the work of the greatest minds in cancer science and medicine from institutions all over the world. This meeting presents the many scientific discoveries across the breadth of cancer research—from prevention, early detection, and interception; to cancer biology, translational, and clinical studies; to survivorship, population science, and advocacy. This year's program, with the theme of "Turning Science into Lifesaving Care," will be a comprehensive, cutting-edge scientific event that you will not want to miss!

We look forward to seeing you!

AACR.ORG • #AACR20

1901031N